J. Microbiol. Biotechnol. (2004), **14**(1), 29–34

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Characterization of Functional Groups of Protonated *Sargassum polycystum* Biomass Capable of Binding Protons and Metal Ions

YUN, YEOUNG-SANG*

Division of Environmental and Chemical Engineering and Research Institute of Industrial Technology, Chonbuk National University, Chonbuk 561-756, Korea

Received: January 14, 2003
Accepted: November 3, 2003

Abstract Biosorption technology is recognized as an economically feasible alternative for the removal and/or recovery of metal ions from industrial wastewater sources. However, the structure of biosorbents is quite complex when compared with synthetic ion-exchange resins,which makes it difficult to quantify the ion-binding sites. Accordingly, this report describes a well-defined method to characterize the pK values and numbers of biomass functional groups from potentiometric titration data. When the proposed method was applied to *Sargassum polycystum* biomass as a model biosorbent, it was found that the biomass contained three types of functional groups. In addition, the carboxyl group (pK= 3.7±0.09) was found to be the major binding sites (2.57± 0.06 mmol/g) for positively-charged heavy-metal ions.

Key words: Biosorption, functional groups, binding site, potentiometric titration, protons, mathematical modeling

The low cost of biosorbents is a tangible advantage for biosorption over other technologies, such as ion exchange and reverse osmosis, for removal and/or recovery of heavy metals from wastewater [1, 20]. Therefore, new types of biomass have been investigated and modified physically, chemically, and even biologically to improve the metal sequestering performance [1, 3, 12- 14, 19].

However, the complex nature of the functional groups of natural or modified biosorbents impedes the current understanding of the metal binding phenomena and the mechanistic modeling of the sorption processes. Numerous chemical groups have been proposed to contribute to metal biosorption, such as carboxyl, carbonyl, sulfonate, sulfhydryl, phosphonate, and hydroxyl groups [5, 7, 10, 11, 24]. These functional groups depend upon certain kinds of biosorbents, thus, identification of the metal-binding functional groups of a given or chosen biosorbent is crucial, because the elucidation of a mechanism and performance modeling of metal biosorption must be based on understanding the interactions between the functional groups and the metal ions.

Accordingly, the current study presents a systematic method to identify the functional groups and quantify their pK values and contents. As a model biosorbent, the biomass of the brown seaweed *Sargassum polycystum* was used. Since the biomass of seaweed is generally large in size, there was no need to separate the solid biosorbents from the wastewater after treatment.

*Corresponding author
Phone: 82-63-270-2308; Fax: 82-63-270-2306;
E-mail: ysyun@chonbuk.ac.kr

Materials and Methods

Biosorbents

The brown seaweed *S. polycystum* was obtained from Cebu, The Philippines (courtesy of MCPI Inc). The sun-dried biomass was treated with 0.5 N HNO_3 solution, thereby replacing the natural mix of ionic species with protons. The acid-treated biomass, designated as the protonated biomass in the current study, was washed several times with deionized distilled water and then dried at 60°C in an oven for 24 h. The resulting dried *Sargassum* biomass was used as the biosorbent in all subsequent experiments.

Chemicals

All chemicals used in this study were of analytical grade. $LiNO_3$ was used to change the ionic strength. In order to adjust the pH, concentrated $LiOH{\cdot}H_2O$ and HNO_3 solutions were used. Although Na^+ is generally present in aquatic systems, it can cause alginate leaching from the biomass matrix at elevated concentrations [7, 8]. Therefore, to avoid any additional complexity (i.e., biomass leaching effect), Li^+ which does not induce such an effect, was used as the background and/or competing light ion. NO_3^-, Cl^-, and SO_4^{2-} are common anions in metal-bearing effluents.

However, as predicted using a thermodynamic database such as MINEQL+ [17], Cl^- tends to form a complex with metal ions (e.g., $CdCl^+$) and SO_4^{2-} can cause precipitation (e.g., $CdSO_2$) as well as complexation [e.g., $Cd(SO_4)_2^{2-}$] to a varying degree, depending on its concentration and the solution pH. Charge alteration due to complexation is already known to affect the performance of metal biosorption [22, 24]. Since none of these ions were necessary for the scope of this study, only NO_3^- was used as the ionic background.

Potentiometric Titration Experiments

The potentiometric titration was carried out with 5 g/l of biomass concentration at two different ionic strengths (0.1 and 1 mM). Forty-ml plastic bottles (high-density polyethylene) were used for the titration experiments. First, the biomass and 20 ml of water (CO_2-free) of the desired ionic strength were placed in each bottle. CO_2-free water was obtained by stripping water with nitrogen gas for 2 h with vigorous mixing. Different volumes of 1 N LiOH or 1 N HNO_3 were added to each bottle containing the biomass suspension. After closing the bottle caps, the bottles were agitated using a shaker (200 rpm) at room temperature for 24 h. Preliminary tests showed that 24 h was sufficient to achieve the proton sorption equilibrium. Thereafter, the equilibrium pH was measured using an electrode (Ingold). The control titration experiments with water solution of the same ionic strength were carried out without the biomass in order to compare them with the titration data when the biomass was present. During the titration experiments, the CO_2-free condition was maintained to avoid the influence of inorganic carbon on the solution pH.

Parameter Estimation

The parameters of the proton-binding model were obtained by fitting the model to the potentiometric titration data using the Marquardt-Levenberg nonlinear regression algorithm [16]. The computer software Sigma Plot (version 4.0, SPSS, U.S.A.) was used for the nonlinear regression.

RESULTS AND DISCUSSION

Potentiometric Titration of Biomass

The biomass titration curve displayed its distinct characteristics depending on the type and amount of functional groups present in the biomass (Fig. 1). When increasing the base addition, the final pH also increased. However, due to the buffering capacity of the biomass, the titration curve of the biomass suspension was quite different from that of the control solution without the biomass. In order to confirm the reproducibility of the titration curve, duplicate experiments were carried out showing enough consistency, as seen in Fig. 1. However, at pH>6, certain deviations were found,

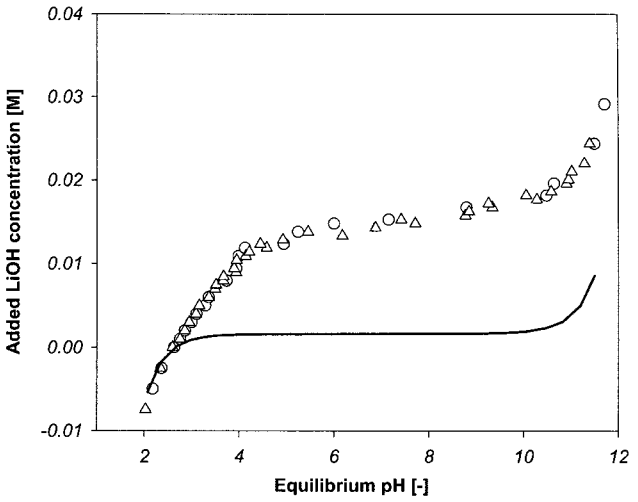

Fig. 1. Potentiometric titration of protonated *Sargassum polycystum* biomass.
The biomass concentration and initial ionic strength were 5 g/l and 0.1 M, respectively. The experiments were duplicated: (○) first trial, (△) second trial. The line represents the titration result for water containing 0.1 M of ionic strength without the biomass.

probably due to the leaching of the biomass constituents, known to be considerable with an increasing pH [8].

Each data point in Fig. 1 was obtained from different bottles. The total initial amount of protons ($TOTH_i$) is the sum of the protons in the added bases or acids ($C_B - C_A$) and of the protons in the biomass suspension ($[H^+]_i - [OH^-]_i + Xq_{H,i}$) as follows:

$$TOTH_i = [H^+]_i - [OH^-]_i + Xq_{H,i} + (C_B - C_A)_B + Y_i \quad (1)$$

where subscript i represents the initial state, X is the biomass concentration, and $q_{H,i}$ stands for the proton uptake equilibrating with $[H^+]_i$ in the solution. C_B and C_A are the concentrations of the added base and acid, respectively. Subscript B in $(C_B - C_A)_B$ represents the system containing the biomass. In this study, the effects of acidic or basic impurities possibly present in the chemicals used and on the vessel wall were expressed using the term Y-, representing unknown constituents that can react with H^+ or OH^-. During the contact between the biomass, the solution, and base added, protons were released from the protonated biomass, and the system eventually reached an equilibrium state. The total protons in the final equilibrium state (subscript f) can be expressed as follows:

$$TOTH_f = [H^+]_f - [OH^-]_f + Xq_{H,f} + Y_f \quad (2)$$

Since there is neither input nor output through the system boundary (CO_2-free condition), the total protons in the final state ($TOTH_f$) should be identical to those in the initial state. Therefore, subtracting equation (1) from equation (2) yields

$$(C_B - C_A)_B = ([H^+]_i - [H^+]_f) - ([OH^-]_i - [OH^-]_f) + X(q_{H,i} - q_{H,f}) + (Y_i - Y_f) \quad (3)$$

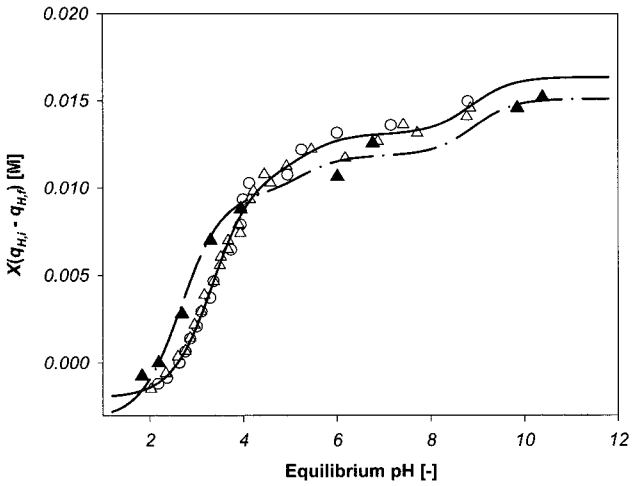

Fig. 2. Proton release $[X(q_{H,i}-q_{H,f})]$ during titration of biomass (5 g/l) at different ionic strengths.
The initial ionic strengths were 0.1 M (○: first trial, △: second trial) and 1 M (▲). The lines were produced using the proton biosorption model equations (5) and (16)] with the model parameters (Table 1).

In the case of the water titration without the biomass, but with the same initial ionic strength (I_i), there was no biomass effect. Therefore:

$$(C_B-C_A)_W=([H^+]_i-[H^+]_f)-([OH^-]_i-[OH^-]_f)+(Y_i-Y_f) \quad (4)$$

where subscript w indicates the water titration without the biomass. When the initial pH of the water titration was adjusted to that of the biomass titration (i.e., pH 2.63 in the case of I_i=0.1 M, as shown in Fig. 1), subtracting equation (4) from equation (3) gives a simple relationship between $\Delta(C_B-C_A)$ and the proton release $(q_{H,i}-q_{H,f})$:

$$\Delta(C_B-C_A)=(C_B-C_A)_B-(C_B-C_A)_W=X(q_{H,i}-q_{H,f}) \quad (5)$$

Figure 2 shows the plot of $X(q_{H,i}-q_{H,f})$ versus the final pH at different ionic strengths. Here, the data for the high pH range were omitted, because the added base concentration significantly changed (>10%) the ionic strength. As seen in Fig. 2, an apparent inflection point was observed around pH 3.5, indicating the existence of a functional group with a pK_H value between pH 3 and 4. The negative value of $X(q_{H,i}-q_{H,f})$ for the low pH range reflects that the acid was added to achieve such a low pH, and that the protons were taken by the biomass. When there was no addition of base or acid ($q_{H,i}=q_{H,f}$), the equilibrium pH at I_i=1 M was significantly lower than that at I_i=0.1 M: the higher the $LiNO_3$ concentration used, the more protons were released. This reflects that Li^+ and/or the ionic strength potentially interfered with the proton binding to the biomass, although this effect was not significant when compared with the effect of the pH.

Modeling of Proton Biosorption

In order to describe the titration curves (Fig. 2), the proton uptake (q_H) was modeled as a function of the sorption system pH and of the background ion present (Li^+ in the present study). It was also assumed that the biomass contained some negatively and/or positively charged groups (on the basis of the neutral condition) capable of binding the protons.

Supposing a certain negatively-charged group ($^iB^-$), its reaction with a proton and its related equilibrium constant (^iK_H) can be defined as follows:

$$^iBH=^iB^-+H^+ \qquad ^iK_H=\frac{[^iB^-]\gamma\{H^+\}}{[^iBH]} \quad (6)$$

where [ ] and { } indicate the concentration and activity, respectively. The activity coefficient (γ) of the monovalent ion (here $^iB^-$) is expressed by Davies' equation [6]:

$$\log_{10}\gamma=-Az^2\left(\frac{\sqrt{I}}{1+\sqrt{I}}-bI\right) \quad (7)$$

where the constants A and b had values of 0.5 and 0.24, respectively, as chosen from the software MINEQL+ [17], Z is the ion valence, and I stands for the ionic strength defined as $I=0.5\sum z_i^2C_i$, where C_i is the concentration of any ionic species in the system.

Furthermore, the negatively-charged group was assumed to be associated with the other cation Li^+, as seen in the earlier studies [4, 21]. This premise was based on the reports that even light ions can be bound to the biomass, although very weakly through the ion-exchange mechanism [4, 15]. The association of Li^+ with the negatively-charged group is expressed as follows:

$$^iBH+Li^+=^iBLi+H^+ \qquad ^iK_{Li}=\frac{[^iBLi^-]\{H^+\}}{[^iBH][Li^+]\gamma} \quad (8)$$

The total concentration of the functional group is equal to the sum of free ($^iB^-$) and occupied (^iBH or ^iBLi) sites as follows:

$$[^iB]_T=[^iB^-]+[^iBH]+[^iBLi]$$
$$=[^iB^-]\left(1+\frac{\{H^+\}\gamma}{^iK_H}+\frac{^iK_{Li}[Li^+]\gamma^2}{^iK_H}\right) \quad (9)$$

Therefore

$$[^iB^-]=\frac{^ibX}{1+\frac{\{H^+\}\gamma}{^iK_H}+\frac{^iK_{Li}[Li^+]\gamma^2}{^iK_H}} \quad (10)$$

where the total concentration of the functional group was expressed by the weight-specific number of the functional group times the biomass concentration (g/l). Therefore, the proton uptake (^iq_H) and the Li uptake ($^iq_{Li}$) by the functional group can be the functions of the respective proton and Li concentrations and their activity coefficients:

$$^iq_H=\frac{[^iBH]}{X}=\frac{^ib\{H^+\}\gamma}{^iK_H+\{H^+\}\gamma+^iK_{Li}[Li^+]\gamma^2} \quad (11)$$

$$ {}^{i}q_{Li}=\frac{[{}^{i}BLi]}{X}=\frac{{}^{i}b\,{}^{i}K_{Li}\{Li^{+}\}\gamma^{2}}{{}^{i}K_{H}+\{H^{+}\}\gamma+{}^{i}K_{Li}[Li^{+}]\gamma^{2}} \quad (12) $$

In addition, a certain positively-charged functional group (${}^{j}BH_2^{+}$) may also be present in the biomass under neutral conditions (for example, NH_3^{+}), and its reaction with the protons can be expressed as follows:

$$ {}^{j}BH_2^{+}={}^{j}BH+H^{+} \qquad {}^{j}K_{H}=\frac{[{}^{j}BH]\{H^{+}\}}{[{}^{j}BH_2^{+}]\gamma} \quad (13) $$

However, the positively-charged group cannot be associated with the Li^{+} cation. Since $[{}^{j}BH]={}^{j}K_{H}[{}^{j}BH_2^{+}]\gamma/\{H^{+}\}$ from equation (13), the total sites become

$$ [{}^{j}B]_{T}=[{}^{j}BH_2^{+}]+[{}^{j}BH]=[{}^{j}BH_2^{+}]\left(1+\frac{{}^{j}K_{H}\gamma}{\{H^{+}\}}\right) \quad (14) $$

Therefore, the protons binding to this functional group can be expressed as follows:

$$ {}^{j}q_{H}=\frac{[{}^{j}BH_2^{+}]}{X}=\frac{{}^{j}b}{1+{}^{j}K_{H}\gamma/\{H^{+}\}} \quad (15) $$

Thus, the total uptake of protons by the biomass is the sum of the protons taken up by all kinds of negative and positive groups:

$$ q_{H}=\sum_{i=1}\frac{{}^{i}b\{H^{+}\}\gamma}{{}^{i}K_{H}+\{H^{+}\}\gamma+{}^{i}K_{Li}[Li^{+}]\gamma^{2}}+\sum_{j=1}\frac{{}^{j}b}{1+{}^{j}K_{H}\gamma/H^{+}} \quad (16) $$

The total uptake of Li by the biomass (only by negatively-charged groups) can also be obtained in the same manner:

$$ q_{Li}=\sum_{i=1}\frac{{}^{i}b\,{}^{i}K_{Li}\{Li^{+}\}\gamma^{2}}{{}^{i}K_{H}+\{H^{+}\}\gamma+{}^{i}K_{Li}[Li^{+}]\gamma^{2}} \quad (17) $$

Since the proton uptake [equation (16)] was modeled as a function of the pH ($=-\log_{10}\{H^{+}\}$) and concentration of background Li^{+} cations, equation (5) could be simultaneously fitted to all the titration curves (Fig. 2) obtained at the two ionic strengths (or Li concentrations). It was assumed that $[Li^{+}]$ was constant at the initial concentration for all titration bottles, because the initial Li concentration was high (0.1 and 1 M), making the change of Li concentration due to LiOH addition negligible. Furthermore, since the affinity of light ions to a seaweed biomass is generally very weak when compared with protons or heavy-metal ions [4, 25], the Li uptake was not expected to significantly affect the Li concentration, which was later confirmed (Fig. 3).

As a result, the three-site model (two types of negative groups and one type of positive group) was able to completely describe the titration curves (Fig. 2), whereas two- or one-site functional group models were unable to describe the titration data, especially at a high pH (data not shown). As can be seen in Fig. 2, the proton biosorption model successfully describes the titration data according to the Li concentration as well as the solution pH. The estimated parameters are summarized in Table 1.

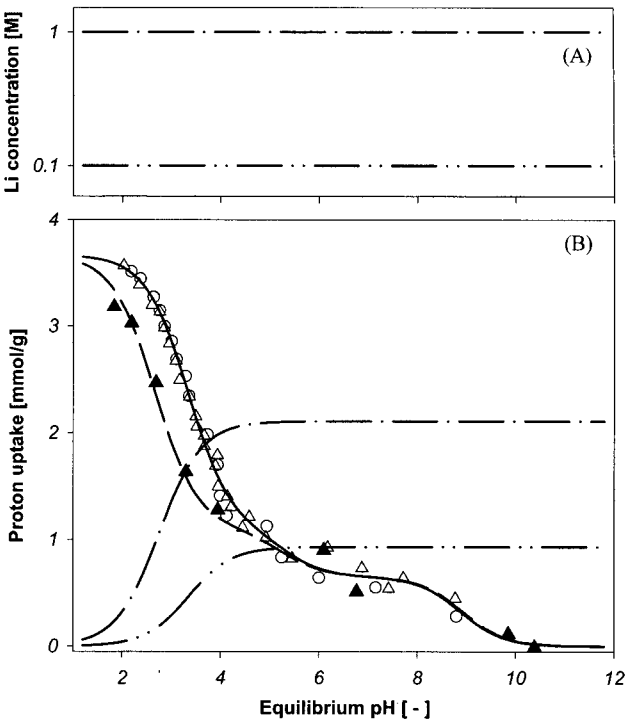

Fig. 3. Uptake of proton and lithium relative to equilibrium pH and ionic strength.

The biomass concentration was 5 g/l and the initial ionic strengths were 0.1 M (○: first trial, △: second trial) and 1 M (▲). The lines were produced using the developed model [equation (16) for the proton uptake, equation (17) for the lithium uptake, and lithium mass balance for the final lithium concentration]. Final lithium concentration (A): -·- 0.1 M and —— 1 M of initial ionic strength. Proton uptake (B): — 0.1 M and ---- 1 M of initial ionic strength. Lithium uptake (B): -·-· 0.1 M and -·- 1 M of initial ionic strength.

Functional Groups of Biomass

The first group was found to be most abundant (2.57± 0.06 mmol/g) and its pK_H value was estimated at 3.70± 0.09, as expected from the apparent inflection point of Fig. 1. Carboxyl groups in biological polymers have pK_H

Table 1. Dissociation Constants (K_H) and Number (b) for Three Types of Functional Groups in *Sargassum polycystum* Biomass[a].

Functional groups[b]	Charge	pK_H (-)[c]	b (mmol/g)[c]	Li-binding constant (-)[c]
1st group	-	3.70 (0.09)	2.57 (0.06)	2.64 (0.46)
2nd group	-	5.41 (0.31)	0.45 (0.07)	-
3rd group	+	8.77 (0.28)	0.65 (0.11)	-

[a]Coefficient of determination was 0.992.

[b]1st functional group indicates a carboxyl site, 2nd group a possible phosphonate site, and 3rd group an amine or thiol site.

[c]Standard errors of estimated parameters are given in parentheses.

values ranging from 3.5 to 5.0 [10]. Furthermore, the pK_H values of carboxyl groups of alginate, the material mainly responsible for metal sequestering by seaweed [7], are known to range between 3.38 (for mannuronic residues) and 3.65 (for guluronic residues) [9]. Therefore, the first functional group was believed to be the carboxyl group. The pK_H value and number of the second binding sites (negatively charged) were estimated to be 5.41±0.31 and 0.45± 0.07 mmol/g, respectively, suggesting them as phosphonate groups; the phosphonate groups of phospholipids present in the plasma membrane of brown seaweed [18] have a similar range of the pK_H values [10]. The last group (positively charged) was considered to be an amine or thiol group with pK_H values for various biomaterials ranging between 8 and 10 [2, 10]. Negatively-charged carboxyl and phosphonate groups can bind to cationic metal ions. Meanwhile, positively-charged amine groups can play a role in binding anionic metal complexes, like oxometal ions. These aspects are studied and discussed in detail in a separate article [23].

Since the third group had positive charge, the Li binding to this group was not expected. Furthermore, although the phosphonate group had a potential to bind Li, the binding constant ($^2K_{Li}$) was estimated to be nearly zero (8.67×10^{-13}) with a large standard error (2.83×10^{-5}), indicating that the Li binding to the phosphonate group was indeed negligible.

Proton Content of Protonated Biomass

The proton content of the protonated biomass ($q_{H,o}$) was evaluated with the help of the proton biosorption model. After the protonated biomass was soaked in water containing different ionic strengths (0, 0.01, 0.1, 1 M $LiNO_3$), the equilibrium pH was measured under CO_2-free conditions. As such, in this experiment, the proton quantity of the original state ($Xq_{H,o}+[H^+]_o-[OH^-]_o$) was assumed to be the same as that of the equilibrium state ($Xq_{H,f}+[H^+]_f-[OH^-]_f$). In addition, because $[H^+]_o\cong[OH^-]_o$ and $[OH^-]_f\ll[H^+]_f$, the proton content of the protonated biomass was obtained according to $q_{H,o}\cong q_{H,f}+[H^+]_f/X$, where $q_{H,f}$ was calculated using the model [equation (16)], while $[H^+]_f$ was directly measured. As a result, the proton content of the protonated biomass was estimated at 3.66±0.40 mmol/g, indicating that the protonated biomass was almost saturated with protons when compared with the total binding sites (3.67± 0.24 mmol/g).

Li Interaction with Proton Binding

The proton uptake relative to the pH and Li concentration is shown in Fig. 3 together with the model output. When the pH decreased below pH 2, the proton uptake reached the maximum capacity (3.67 mmol/g). The higher the ionic strength (or Li concentration), the more protons were required for the maximum uptake. This was mainly due to the interference of Li with the proton binding. As can be seen in Fig. 3, the Li uptake predicted by equation (17) increased with a higher Li concentration and the pH. However, as expected, at an elevated pH (>4.5), the Li uptake became independent of the pH, since apparently only the Li binding occurred with the carboxyl group.

The equilibrium constant ($^1K_{Li}=2.64\pm0.46\times10^{-3}$) for the Li binding to the carboxyl group [equation (8)] corresponded to the selectivity coefficient of the Li-H ion exchange. Therefore, the affinity of Li for the biomass was relatively low, approximately 0.26% of that displayed by the protons. The reason why the Li uptake was considerable at pH>4.5 ($[H^+]<3.2\times10^{-5}$ M) was because the Li concentration was fairly high ($[Li^+]$=0.1 or 1 M). However, the same reasons can also explain why the Li concentration remained constant at its initial concentration (within 0.4% error for I_i=1 M, and 0.2% for I_i=0.1 M), reflecting that the assumption made in the parameter estimation procedure was reasonable.

From Fig. 3, when a high concentration of Li (and probably other light ions) was present, the competition between Li and the protons for the carboxyl group was evident, even though the Li affinity was far lower than that for the protons. In particular, at an elevated pH (up to pH 6), Li was significantly competitive, because of the lack of protons. In addition to the competition for the binding sites, the background ions (Li^+ and NO_3^- in the current study) also had the ability to disturb the proton uptake via reducing the activities of the binding sites and protons.

Acknowledgments

This work was supported by the Korea Science & Engineering Foundation (KOSEF) through the AEBRC at POSTECH and partially by grant No. R08-2003-000-10987-0 from the Basic Research Program of KOSEF.

GLOSSARY

- [ ] : Representative concentration for bracketed species (mol/l)
- { } : Representative activity for bracketed species (mol/l)
- ^iBH : i-th negatively-charged functional group
- ^jBH : j-th positively-charged functional group
- ^ib : Weight-specific number of i-th negatively-charged functional group (mol/g)
- ^jb : Weight-specific number of j-th positively-charged functional group (mol/g)
- B : Representative functional group in biomass
- C_B, C_A : Concentrations of added base and acid, respectively (mol/l)
- $[H^+]_f$: Proton concentration in final state with equilibrated system (mol/l)

$[H^+]_i$: Proton concentration in initial state (i.e., in stock solution) when biomass and LiOH solution were mixed together and equilibrated (mol/l)
$[H^+]_o$: Proton concentration in original state before reactants were mixed (mol/l)
I : Ionic strength (mol/l)
I_i : Initial ionic strength (mol/l)
${}^iK_H$: Proton binding constant for *i*-th negatively-charged functional group
${}^jK_H$: Proton binding constant for *j*-th positively-charged functional group
${}^iK_{Li}$: Li binding constant for *i*-th negatively-charged functional group
q_H : Total uptake of protons by biomass (mol/g)
$q_{H,f}$: Uptake of protons in final state (mol/g)
$q_{H,i}$: Uptake of protons in initial state (mol/g)
$q_{H,o}$: Proton uptake by protonated biomass (mol/g)
q_{Li} : Total uptake of Li by biomass (mol/g)
TOTH : Total proton concentration in system (mol/l)
$TOTH_f$: Total proton concentration in final state (mol/l)
$TOTH_i$: Total proton concentration in initial state (mol/l)
V : Solution volume (l)
X : Concentration of biomass (g/l)
z : Valence of ion (-)
γ : Activity coefficient of monovalent ion (-)

REFERENCES

1. Bailey, S. E., T. J. Olin, R. M. Bricka, and D. D. Adrian. 1999. A review of potentially low-cost biosorbents for heavy metals. *Water Res.* **33:** 2469-2479.
2. Buffle, J. 1988. *Complexation Reactions in Aquatic Systems: An Analytical Approach*, pp. 156-157, pp. 280-283, p. 323. Ellis Horwood, Chichester, U.K.
3. Cho, J. S., J.-S. Hur, B.-H. Kang, P.-J. Kim, B.-K. Sohn, H.-J. Lee, Y.-K. Jung, and J.-S. Heo. 2001. Biosorption of copper by immobilized biomass of *Pseudomonas stutzeri*. *J. Microbiol. Biotechnol.* **11:** 964-972.
4. Crist, R. H., J. R. Martin, D. Carr, J. R. Watson, and J. Clarke. 1994. Interaction of metals and protons with algae: 4. Ion exchange vs adsorption models and a reassessment of scatchard plots; ion exchange rates and equilibria compared with calcium alginate. *Environ. Sci. Technol.* **28:** 1859-1866.
5. Crist, R. H., K. Oberholser, N. Shank, and M. Nguyen. 1981. Nature of bonding between metallic ions and algal cell walls. *Environ. Sci. Technol.* **15:** 1212-1217.
6. Davies, C. W. 1962. *Ion Association*. Butterworth, Washington, DC, U.S.A.
7. Fourest, E. and B. Volesky. 1995. Contribution of sulfonate groups and alginate to heavy metal biosorption by the dry biomass of *Sargassum fluitans*. *Environ. Sci. Technol.* **30:** 277-282.
8. Fourest, E. and B. Volesky. 1997. Alginate properties and heavy metal biosorption by marine algae. *Appl. Biochem. Biotechnol.* **67:** 215-226.
9. Haug, A. 1961. The affinity of some divalent metals to different types of alginate. *Acta Chem. Scand.* **15:** 1794-1795.
10. Hunt, S. 1986. Diversity of biomass structure and its potential for ion-binding applications, pp. 15-46. *In* H. Eccles and S. Hunt (eds.), *Immobilization of Ions by Biosorption*. Ellis Horwood, Chichester, U.K.
11. Jeon, C., J. Y. Park, and Y. J. Yoo. 2001. Biosorption model for binary adsorption sites. *J. Microbiol. Biotechnol.* **11:** 781-787.
12. Jeon, C., J. Y. Park, and Y. J. Yoo. 2002. Characteristics of metal removal using carboxylated alginic acid. *Water Res.* **36:** 1814-1824.
13. Kim, D. W., D. K. Cha, H. J. Seo, and J. B. Bak. 2002. Influence of growth rate on biosorption of heavy metals by *Nocardia amarae*. *J. Microbiol. Biotechnol.* **12:** 878-881.
14. Kim, S. K., C.-G. Lee, and H. S. Yun. 2003. Heavy metal adsorption characteristics of extracellular polysaccharide produced by *Zoogloea ramigera* grown on various carbon sources. *J. Microbiol. Biotechnol.* **13:** 745-750.
15. Lee, H. S. and B. Volesky. 1997. Interaction of light metals and protons with seaweed biosorbent. *Water Res.* **31:** 3082-3088.
16. Mardquardt, D. W. 1963. An algorithm for least square estimation of parameters. *J. Soc. Ind. Appl. Math.* **11:** 431-441.
17. Schecher, W. D. 1991. *MINEQL+: A Chemical Equilibrium Model for Personal Computers, Users Manual Version 2.22*. Environmental Research Software, Inc., Hallowell, ME, U.S.A.
18. Schiewer, S. and B. Volesky. 2000. Biosorption processes for heavy metal removal, pp. 329-362. *In* D. R. Lovley (ed.), *Environmental Microbe-Metal Interactions*. ASM Press, Washington, DC, U.S.A.
19. Sousa, C., A. Cebolla, and V. Lorenzo. 1996. Enhanced metalloadsorption of bacterial cells displaying poly-His peptides. *Nature Biotechnol.* **14:** 1017-1020.
20. Volesky, B. 1990. Removal and recovery of heavy metals by biosorption, pp. 7-43. *In* B. Volesky (ed.) *Biosorption of Heavy Metals*. CRC Press, Boca Raton, FL, U.S.A.
21. Westall, J. C., J. D. Jones, G. D. Turner, and J. M. Zachara. 1995. Models for association of metal ions with heterogeneous environmental sorbents: 1. Complexation of Co(II) by leonardite humic acid as a function of pH and $NaClO_4$ concentration. *Environ. Sci. Technol.* **29:** 951-959.
22. Yang, J. B. and B. Volesky. 1999. Modeling the uranium-proton ion exchange in biosorption. *Environ. Sci. Technol.* **33:** 4079-4085.
23. Yun, Y.-S. and B. Volesky. 2003. Modeling of lithium interference in cadmium biosorption. *Environ. Sci. Technol.* **37:** 3601-3608.
24. Yun, Y.-S., D. Park, J. M. Park, and B. Volesky. 2001. Biosorption of trivalent chromium on the brown seaweed biomass. *Environ. Sci. Technol.* **35:** 4353-4358.
25. Yun, Y.-S., H. Niu, and B. Volesky. 2001. The effects of impurities on metal biosorption, pp. 573-580. *Proc. of the 14th International Biohydrometallurgy Symposium*, Ouro Preto, Brazil.