

Surface Complexation Modeling of Proton and Cd Adsorption onto an Algal Cell Wall

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This study quantifies Cd adsorption onto the cell wall of the algal species *Pseudokirchneriella subcapitata* by applying a surface complexation approach to model the observed adsorption behavior. We use potentiometric titrations to determine deprotonation constants and site concentrations for the functional groups on the algal cell wall. Adsorption and desorption kinetics experiments illustrate that adsorption of Cd onto the cell wall is rapid and reversible, except under low pH conditions. Adsorption experiments conducted as a function of pH and total Cd concentration yield the stoichiometry and site-specific stability constants for the important Cd–algal surface complexes. We model the acid/base properties of the algal cell wall by invoking four discrete surface functional group types, with pK_a values of 3.9 ± 0.3 , 5.4 ± 0.1 , 7.6 ± 0.3 , and 9.6 ± 0.4 . The results of the Cd adsorption experiments indicate that the first, third, and fourth sites contribute to Cd adsorption under the experimental conditions, with calculated log stability constant values of 4.1 ± 0.5 , 5.4 ± 0.5 , and 6.1 ± 0.4 , respectively. Our results suggest that the stabilities of the Cd–surface complexes are high enough for algal adsorption to affect the fate and transport of Cd under some conditions and that on a per gram basis, algae and bacteria exhibit broadly similar extents of Cd adsorption.

Introduction

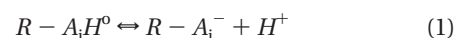
Heavy metal contamination of surface waters is an all too frequent result of accidental or improper waste disposal practices. Surface waters contain significant concentrations of algae and bacteria, both of which have cell walls that contain organic acid functional groups which are capable of adsorbing protons and aqueous metal cations. These adsorption reactions can control buffering behavior and metal speciation in both natural and engineered systems (1, 2). Adsorption of protons and metals onto bacterial cell walls has received considerable attention in recent experimental and modeling studies (3–6), including several studies utilizing site-specific surface complexation approaches. While proton and metal adsorption onto algal cell walls has been studied to some extent (7, 8), modeling efforts typically are limited to partition coefficient or other isotherm approaches, which offer less flexibility than surface complexation models in extrapolating experimental data to complex realistic settings.

Recent work has shown that surface complexation modeling can successfully account for competitive proton and

metal adsorption onto bacterial surfaces and can account for changes in pH and bacteria:metal ratio on metal adsorption behavior (4, 6, 9, 10). Therefore, our objectives were to test if such an approach is applicable to algal surfaces, and if so, to use acid/base titrations and metal adsorption experiments to constrain the thermodynamic properties that represent the foundation of the surface complexation modeling approach. Specifically, we chose to measure and model the interactions between Cd and the green alga *Pseudokirchneriella subcapitata*. We chose to study Cd not only because of its human health and environmental effects but also because the aqueous chemistry of Cd is relatively simple, with Cd^{2+} being the dominant aqueous species to approximately pH 9.5. *P. subcapitata* was chosen for these experiments because it is commonly found in surface waters and as such has been well-studied, particularly in a range of toxicity studies (11–13).

Surface Complexation Modeling

We apply a similar surface complexation modeling approach to account for algal adsorption of protons and metal cations to that applied by Fein et al. (4, 14) to bacterial adsorption. That is, we model the acidity of surface functional groups via deprotonation reactions:



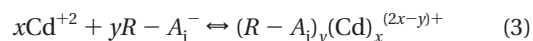
where R and A_i represent the algal cell wall and a functional group type, respectively. The distribution of protonated and deprotonated functional group sites is quantified via mass balance equations, such as

$$K_a = \frac{[R - A_i^-] a_{H^+}}{[R - A_i H^0]} \quad (2)$$

where K_a represents the acidity constant, a represents the activity of the subscripted species, and the brackets represent the concentration of surface sites in moles per liter of solution. Activities of all surface species are assumed to be equal to unity.

In applying this approach to modeling the surface acidity of algae, we implicitly assume that the deprotonation of each type of functional group, A_i , can be represented as a single deprotonation of an organic acid. Because all of our experiments were conducted at the same ionic strength, we ignore potential ionic strength effects on the surface electric field, applying a nonelectrostatic model to account for the titration data. Potentiometric titration experiments are essentially studies of proton adsorption and desorption, yet because the solvent contains the same element as is reacting with the surface of interest, it is impossible to apply a traditional mass balance approach. Instead, one must define a zero proton condition for the algal cell wall and account for changes in proton concentrations relative to that condition (15). After the approach by Fein et al. (14), we choose the fully protonated algal cell wall to represent our zero proton condition, and we use FITEQL to solve for the initial state of protonation in each titration (15).

We represent interactions between aqueous Cd^{2+} and deprotonated algal surface sites as follows:



where $(R - A_i)_y (Cd)_x^{(2x-y)+}$ represents the Cd–algal surface complex, and x and y are stoichiometric coefficients to be

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determined experimentally. The mass balance equation for reaction 3 is

$$K_{\text{ads}} = \frac{[(R - A_i)_y(\text{Cd})_x]^{(2x-y)+}}{[\text{Cd}^{+2}]^x[\text{R} - \text{A}_i^-]^y} \quad (4)$$

where K_{ads} is the thermodynamic equilibrium constant for reaction 3. Acid/base potentiometric titration data provide constraints on the number of site types, their K_a values, and their site concentrations; metal adsorption measurements conducted as a function of pH constrain the number of sites involved in metal binding, the pH range of influence, and the stability constants for the important metal–algal surface complexes; and metal adsorption isotherms conducted as functions of metal concentration provide constraints on the stoichiometry of reaction 3. We use the program FITEQL 2.0 (16) for the equilibrium thermodynamic modeling of the adsorption data, using aqueous Cd hydrolysis equilibrium constants from Baes and Mesmer (17).

Experimental Procedures

Algal Growth and Preparation. The algae *Pseudokirchneriella subcapitata*, formally known as *Selenastrum capricornutum*, was obtained from the Culture Collection of Algae at the University of Texas at Austin. The green algae were originally isolated in 1959 from the Nitelva River in Akershus, Norway. The algae were cultured in Bristol's solution and medium in 3-L Erlenmeyer flasks for 2–3 weeks at approximately 25 °C (18). The cells were grown under a 120 W plant light on a 16 h light/8 h dark cycle. Cells were washed five times in 0.1 M NaClO₄ prior to experimentation, with each wash supernatant separated by centrifugation at 8000 rpm (or 7150g) for 10 min. After the final rinse, the algal suspension was centrifuged twice at 8000 rpm for 30 min, and excess solution was decanted after each centrifugation to obtain the wet weight of the algal cells. This procedure ensured the removal of easily exchangeable growth medium or exudate components from the cell wall. We confirmed the viability of washed algal cells by resuspending some washed cells in fresh growth medium. The washed cells were found to grow at a rate comparable to unwashed algal cells.

Potentiometric Titrations. Acid/base potentiometric titrations were conducted by first suspending a known wet weight of algal cells (approximately 60–70 g wet weight/L) in 0.1 M NaClO₄, which had been purged of dissolved CO₂ by having N₂ gas bubbled through it for 45–60 min. Four titrations were performed, using algal cells from different growth cultures. Each titration was conducted using a sealed titration vessel in which a positive pressure headspace of N₂ gas was maintained to exclude atmospheric CO₂ from the experimental systems. The titrations were conducted using an automated buret assembly with aliquots of 0.1028 N HCl or 0.1001 N NaOH titrant added to solution after a potentiometric stability of at least 0.1 mV/s was obtained. Utilizing the autotitrator, we first acidified the algal suspension to approximately pH 3. Then, we basified the suspension to approximately pH 10 while measuring the pH change associated with each acid or base addition. We use only the base titration results for thermodynamic modeling.

Metal Adsorption/Desorption Experiments. All metal uptake experiments were conducted as batch experiments in polypropylene test tubes or glass beakers using periodic additions of minute volumes (<1% of total volume) of 0.1 M NaOH and 0.1 M HNO₃ to maintain pH but not affect ionic strength. All experiments were conducted in 0.1 M NaClO₄ with approximately 10 g (wet weight)/L of *P. subcapitata*. NaClO₄ was used as the background electrolyte to minimize aqueous complexation between the electrolyte anion and Cd²⁺. Although the experimental algal concentration is

approximately 1000 times that typically found in oligotrophic lakes, this algal concentration is comparable to that used in biosorption remediation processes, and the molar ratio of algal cell wall sites to total Cd is comparable to that found in systems of environmental interest (19). Each algal suspension was basified to between pH 6 and 7 to prevent acid shock of the cells when a small volume of 1000 parts per million (ppm) Cd aqueous standard solution was added to achieve a total Cd concentration of 10 ppm in all experiments (except the isotherm experiments). At 10 ppm total Cd, none of the experimental solutions were saturated with respect to a Cd-hydroxide solid phase over the experimental pH range, so the removal of Cd from solution during the experiments could be ascribed entirely to algal cell wall adsorption. Floating magnetic stir bars in the isotherm experiments and a rotating wheel in the other adsorption experiments vigorously mixed all algal/metal suspensions. After equilibrium was obtained, the pH of the batch system was measured. Sampling involved the removal of a homogeneous sample from each system, centrifugation of the sample at 8000 rpm for 2 min, and further separation of the solid and solution via a 0.45- μm filter. The filtrate from each sample was acidified with 10 μL of concentrated HNO₃ to prevent metal precipitation.

Adsorption and desorption kinetics experiments with 10 ppm total Cd were conducted to determine the time required to reach equilibrium in algae/metal systems and to test for adsorption reversibility. Adsorption kinetics experiments were conducted under conditions described above while maintaining a constant pH of 7 through the addition of minute volumes of 0.1 M NaOH or 0.1 M HNO₃ when necessary. Three separate desorption experiments were conducted in which the pH of the algal/metal suspensions were adjusted initially to approximately pH 8.7, and the systems were allowed to equilibrate for 3 h. After this initial adsorption period, the pH was lowered to either 3.7, 5.8, or 7.0. Finally, samples were taken as a function of time from the pH decrease as described above.

The Cd adsorption experiments were conducted as a function of pH, from pH 3 to 10, and as a function of total Cd concentration at pH 7 \pm 0.2 with Cd concentrations ranging from 3 to 100 ppm. Periodically, minute volumes of 0.1 M NaOH and 0.1 M HNO₃ were added to the algal/metal suspensions to maintain a constant pH. The results of the adsorption kinetics experiments (see below) indicated that an adsorption equilibrium state is reached within 3 h of contact, so the pH and Cd isotherm adsorption experiments were allowed to equilibrate for 3 h as well.

All aqueous samples were analyzed for total dissolved Cd concentration using a Perkin-Elmer Optima 2000 DV ICP-OES system, operated at a wavelength of 228.802 nm. Along with analysis of each experimental sample, a solution blank, four standards covering the probable range in aqueous Cd concentrations, and known standards interspersed among the samples for error calculation were also analyzed. Both the solution blank and the Cd standards were made using 0.1 M NaClO₄ as a background electrolyte to avoid matrix effects on the analysis. Analytical uncertainty was approximately $\pm 2\%$. The concentration of adsorbed Cd was determined by the difference between the initial total Cd concentration of the system and the measured final Cd concentration in solution.

Results

P. subcapitata cells display a significant buffering capacity over the entire pH range of this study (Figure 1), similar to the pH range of the buffering behavior of fulvic and humic acids, and mineral and bacterial surfaces. However, the algal cells exhibit significantly lower buffering capacity on a per gram basis than do bacterial cells. For example, *Bacillus*

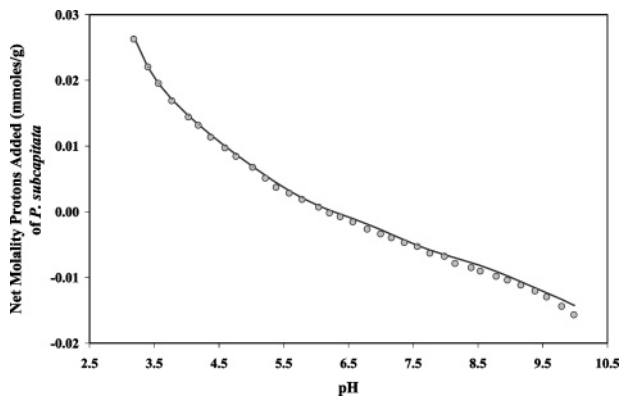


FIGURE 1. Potentiometric titration data (●) for one of the four titrations of *P. subcapitata* in 0.1 M NaClO₄. Results from FITEQL 2.0 modeling are depicted for the best-fitting four-site model.

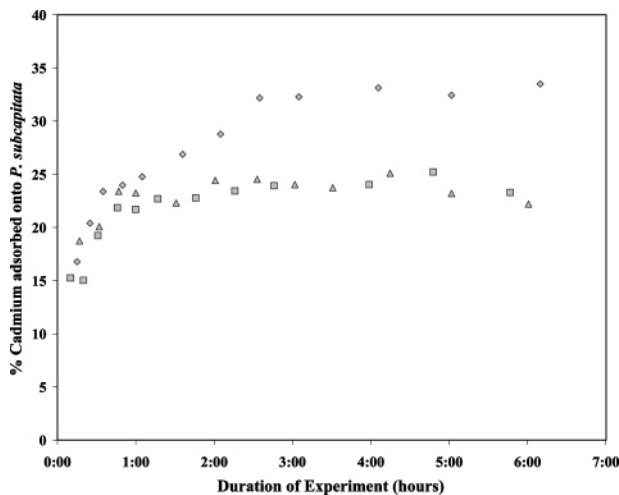


FIGURE 2. Results from adsorption kinetics experiments conducted with a total Cd concentration of 10 ppm. The different data point symbols represent the results from independent batch experiments conducted at a constant pH of 7.0 ± 0.2 . Each experiment began with no adsorbed Cd.

subtilis bacterial cells provide a total buffering capacity of approximately 3.0×10^{-4} mol/g over a pH range of 3–10 in 0.1 M NaClO₄ (14). In contrast, *P. subcapitata* algal cells in 0.1 M NaClO₄ provide a total buffering capacity of approximately 4.2×10^{-5} mol/g over the same pH range.

Results from the adsorption kinetics experiments indicate that Cd adsorption onto the cell wall of *P. subcapitata* reaches steady state within approximately 3 h from the start of the experiment, with the majority of the uptake occurring rapidly during the first hour (Figure 2).

The desorption reaction (Figure 3) is even more rapid, attaining a pH-dependent steady-state value within 1 h, with a maximum change of 7% over the next 6 h. Comparison between the measured extents of desorption and adsorption as a function of pH (Figure 4) suggests that Cd adsorption onto algal cells is reversible under circumneutral pH conditions but is not entirely reversible under lower pH conditions. It is unclear what is responsible for the irreversibility, but it may be due to the effect of acid on the cell wall structure (20).

Our Cd adsorption experiments indicate that solution pH strongly affects the extent of Cd adsorption onto *P. subcapitata* (Figure 4). At low pH, the majority of functional groups are protonated, and minimal adsorption occurs. However, even under the lowest pH conditions studied here, some adsorption occurs, with approximately 10% of the total Cd concentration adsorbing to the cell wall at pH 3. With

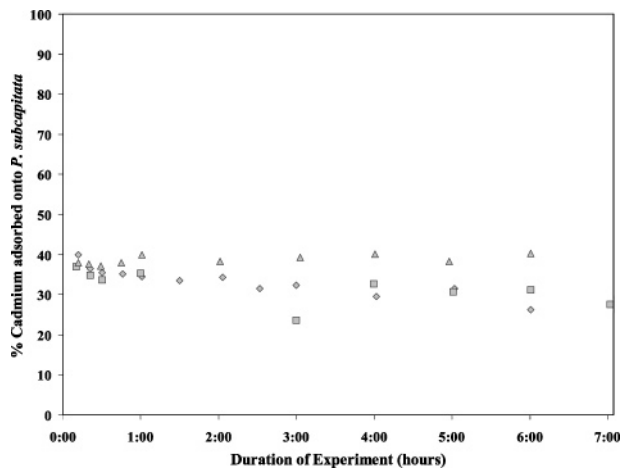


FIGURE 3. Results from desorption kinetics experiments conducted with a total Cd concentration of 10 ppm. Each system was first equilibrated at pH 8.7 to initially adsorb approximately 80% of the total Cd in each case, and the systems were then adjusted to the following final pH values to promote desorption: ▲, pH 7; ■, pH 5.75; and ◆, pH 3.7. The time of contact represents the time since the system pH was lowered to promote desorption. Data are presented in terms of the percentage of total Cd still adsorbed onto the algal cells after the desorption period.

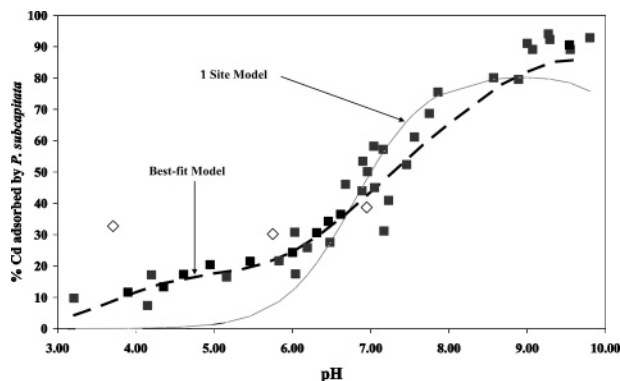


FIGURE 4. Percent of total Cd adsorbed onto *P. subcapitata* for experiments conducted with a total Cd concentration of 10 ppm. Dashed and solid curves represent the best-fitting one-site and three-site models, respectively (see text). Diamonds represent the desorption results.

increasing pH, functional groups on the cell wall deprotonate sequentially, and the extent of cation adsorption increases correspondingly. The greatest increase in Cd adsorption to the cell wall occurs between pH 6 and 8, with the observed Cd adsorption reaching a plateau of 90% adsorption at approximately pH 9.

As shown in Figure 5, the extent of Cd adsorption onto the algal cell wall increases with increasing total Cd concentration in the system and does not reach an adsorption plateau. The lack of an adsorption plateau suggests that, under these experimental conditions, the algal surface is not fully saturated with respect to adsorbed Cd, and hence the adsorption reaction is not site-limited.

Judging from the scatter in the data, the observed experimental uncertainty in the measured extent of Cd adsorption ranges from $\pm 3\%$ at low and high pH to $\pm 9\%$ in the mid-pH range (see Figures 2–4 for a graphical depiction of these uncertainties). These observed uncertainties are significantly larger than those that are typically associated with similar batch adsorption experiments involving metabolically inactive bacterial cells (4, 21, 22). The higher uncertainties associated with these experiments likely result from many factors that were not controlled in these experi-

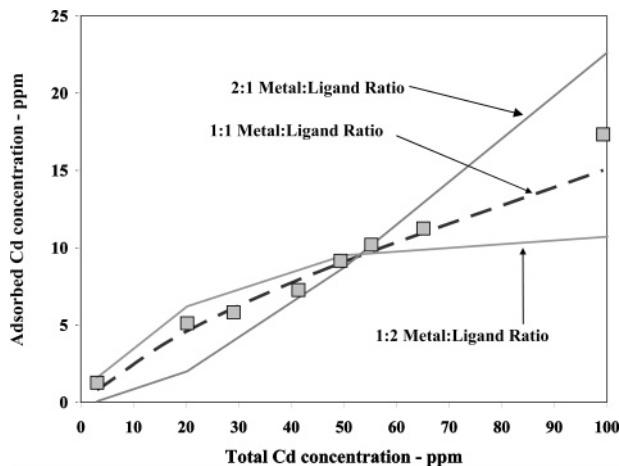


FIGURE 5. Results from the adsorption isotherm experiments conducted at pH 7. The curves represent models constructed using different Cd:Site stoichiometries.

ments, such as cell age, morphology, or metabolic state. For instance, because of the relatively slow growth kinetics for *P. subcapitata*, large quantities of algae were grown at one time, with cells saved from 0 to 21 days before being used in experiments. In addition, some cells that were used in the experiments grew in mats at the bottom of the growth flasks, while other cells grew free in solution, perhaps imparting differing morphologies to the different growth conditions. Another possible explanation for the high uncertainties is that the cells may have undergone some photosynthesis during experimentation, leading to the formation of a proton flux, which altered the protonation state of the cell wall (23) or the buffering capacity of the system.

Discussion

We use FITEQL 2.0 (16) modeling to determine the number of discrete functional group site types that are required to account for the observed algal buffering capacity, attempting to fit one-, two-, three-, four-, and five-site models to the potentiometric titration data. The modeling results, which are shown in Figure 1 for one of the four titrations, indicate that a four-site model yields the best fit to the experimental potentiometric data. In each case, a five-site model does not converge, indicating that the system is underconstrained and that the data do not support a model with five discrete functional group types. In all cases, a four-site model matches the pH dependence of the buffering capacity significantly better than do models with fewer sites. FITEQL calculates a value for a variance function, $V(Y)$, for each data set, with values closest to 1.0 representing the best fits to the data (16). The $V(Y)$ value improves significantly with each additional site considered in the model to a minimum for the four-site model. Average $V(Y)$ values for the four titrations for the one-, two-, three-, and four-site models are 161.1, 19.9, 4.4, and 1.2, respectively. In Table 1, we compile the calculated pK_a values and site concentrations, along with 1σ errors for each value, for the four-site model for each acid/base titration and the average values for all the titrations. We use these average values as a foundation for subsequent modeling of the Cd adsorption data.

A comparison of our modeling results with those for several bacterial species indicates that *P. subcapitata* has fewer total binding sites than are typical on bacterial cell walls. For instance, the total number of binding sites for our algae is 2.77×10^{-5} mol/g, compared to 3.25×10^{-4} and 3.51×10^{-4} mol/g for gram negative bacterial species *Pseudomonas putida* and *Pseudomonas mendocina*, respectively (24), and compared to 3.11×10^{-4} mol/g for gram-positive *B. subtilis* (14). Modeling results of unidentified bacterial

TABLE 1. Potentiometric Titration Modeling Results

titration #	pK_a 's			
1	3.8	5.4	7.7	9.7
2	4.0	5.5	8.0	10.1
3	3.4	5.3	7.5	9.7
4	4.3	5.4	7.2	9.1
average	3.9 ± 0.3	5.4 ± 0.1	7.6 ± 0.3	9.6 ± 0.4

titration #	site concentrations ($\mu\text{mol/g}$)			
1	3.0	10.8	4.1	6.0
2	4.1	9.6	4.5	8.9
3	8.8	10.8	3.7	7.1
4	8.7	8.2	6.6	6.1
average	6.1 ± 3.0	9.8 ± 1.3	4.7 ± 1.3	7.0 ± 1.4

consortia from various uncontaminated and contaminated environments (24, 25) also indicate that the mass normalized functional group concentration on bacterial cell walls is more than an order of magnitude greater than that observed on the algal cell wall in this study. However, it is interesting to note the similarities between the molal ratios of the individual sites for algae and bacteria. The molal ratios of Sites 1–4 for the algae are 1.3:2.1:1:1.5 (normalized to Site 3 concentrations), while those for *B. subtilis* are 1.8:2.5:1:1.7 (14).

The Cd isotherm data (Figure 5) provide constraints on the stoichiometry of Reaction 3, and we use FITEQL 2.0 to test a range of $x:y$ values to account for the observed cadmium adsorption behavior. Because the pH of the isotherm experiments varied only over a small range, the speciation of the algal surface remained constant and these experiments do not place constraints on which site or sites are responsible for the observed adsorption. Therefore, we model the isotherm data by assuming that all adsorbed Cd is located on a generic deprotonated site, with a site concentration equal to the sum of the individual concentrations of the four algal surface sites. This approach assumes that each site type exhibits the same binding stoichiometry. The best-fitting models involving 1:1, 1:2, and 2:1 $x:y$ values are depicted in Figure 5. The 1:1 stoichiometry yields the best fit to the observed adsorption behavior. The 1:2 and the 2:1 $x:y$ models yield calculated adsorption behaviors that are significantly less and more steep, respectively, than that observed in the experiments. On the basis of these results, we conclude that the dominant adsorption reaction involves a 1:1 metal:ligand ratio, and we use this 1:1 stoichiometry to model the adsorption data that were collected as a function of pH.

Because the pH adsorption edge is located between approximately pH 6 and 8, it is most likely that binding of Cd onto Site 3 (with a pK_a of 7.6) is one of the most important adsorption mechanisms responsible for the observed adsorption behavior. Therefore, we first attempt to model the pH data by ascribing Cd binding only onto the deprotonated form of Site 3. As shown in Figure 4 (solid curve), this one-site model provides a reasonable fit to the experimental data in the mid-pH range (between approximately pH 6.5 and 9.0) but significantly underpredicts the observed extent of adsorption both at lower and at higher pH. Model underprediction indicates that additional surface species are involved in the overall Cd adsorption. Including Cd adsorption onto a deprotonated Site 4, along with binding onto deprotonated Site 3, provides a good fit to the high pH data but does not improve the quality of the fit to the low pH measurements (model fit not shown). Including Cd adsorption onto deprotonated Site 2, along with binding onto deprotonated Sites 3 and 4, results in a model which does not converge, indicating a severe misfit between the model

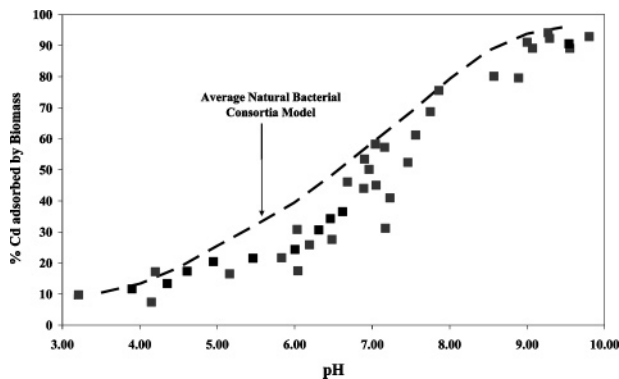


FIGURE 6. Comparison of Cd adsorption onto 10 g (wet mass)/L of *P. subcapitata* measured in this study (filled squares) with the average adsorption behavior determined by Borrok et al. (25) for 10 g (wet mass)/L of bacterial consortia cultured from a range of natural environments (dashed curve). Other than type of microbial cell used, the experimental conditions used in the two studies were identical.

behavior as a function of pH and that of the experimental data. However, a model that involves Cd adsorption onto deprotonated Sites 1, 3, and 4 (dashed curve in Figure 4) represents the best-fitting model to the experimental observations, yielding a significantly better fit than any other 1, 2, 3, or 4 site model. Although the best-fitting models offers a reasonable fit to the data across the pH range of interest, it does slightly underpredict the observed extent of adsorption for the highest pH data. This misfit may indicate the presence of an additional, unaccounted for algal surface complex at high pH, but the resolution and precision of the data are not good enough to rigorously constrain the thermodynamic stability of this putative species.

The calculated log stability constant values for the 1:1 Cd–algal surface complexes from the best-fitting model are 4.1 ± 0.5 , 5.4 ± 0.5 , and 6.1 ± 0.4 for deprotonated Sites 1, 3, and 4, respectively. The stability constants for Cd adsorption onto individual surface site types that we determined for *P. subcapitata* are significantly higher than those determined for comparable site types on cell walls of bacterial consortia grown from a range of natural environments. For instance, the log of the stability constants for Cd binding onto Sites 2, 3, and 4 on the bacterial consortia are 2.7, 4.0, and 5.2, respectively (25). These differences suggest that the algal sites exhibit a significantly higher tendency to adsorb Cd than do the bacterial sites. However, this tendency is offset by the approximately order of magnitude lower total site concentrations found on the algal cell wall compared to bacterial cell walls. Figure 6 compares our experimental measurements of *P. subcapitata* Cd adsorption to the average Cd adsorption edge measured by Borrok et al. (25) for an equal mass of consortia of bacteria cultured from a range of natural environments, using otherwise identical experimental conditions. Although at most pH values Cd adsorption onto *P. subcapitata* is lower than that onto the bacterial surfaces, the extent of adsorption is roughly similar. Our work suggests that on a per mass basis *P. subcapitata* can compete with bacterial cell walls in adsorbing metal and that adsorption onto both types of cell walls must be accounted for when determining the speciation of a metal in a system that contains both bacterial and algal species.

Our experimental data show that algal cell walls exhibit a strong affinity for adsorbing protons and aqueous Cd, suggesting that algal cells can significantly contribute to the buffering behavior and metal speciation in natural and engineered systems. We model the proton adsorption behavior using a four-site discrete pK_a model, similar to that used recently for bacterial surfaces (14, 24, 25). Compared

to bacterial species grown from a range of natural environments, *P. subcapitata* has fewer total binding surface sites, but the individual sites on the algal cell wall exhibit a higher tendency to adsorb Cd that at least partially compensates for the lower site concentrations. The importance of algae in controlling the fate and transport of metals depends on the setting and on the concentration of algae relative to other metal-binding agents, including bacterial and mineral surfaces, and dissolved organic acid anions. Our work demonstrates that the surface complexation model approach provides a means for estimating the importance of algal adsorption on metal speciation and distribution in algal-bearing aqueous systems.

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