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Distribution of protons and Cd between bacterial surfaces and dissolved humic substances determined through chemical equilibrium modeling

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Abstract—Bacteria and dissolved humic substances are capable of binding significant concentrations of metals in natural environments. Recent advances in understanding bacteria-metal and humic-metal complexation have provided a framework for directly comparing the binding capacities of these components. In this study, we use chemical equilibrium modeling to construct an internally consistent set of thermodynamic equilibrium constants for proton and Cd binding onto dissolved humic substances, using a variety of published data sets. Our modeling approach allows for the direct comparison of humic substance binding constants and site densities to those previously published for proton and Cd binding onto natural consortia of bacteria. We then combine these constants into a unified model that accounts for the competition between bacterial surfaces and humic and fulvic acids in order to determine the relative importance of each component on the total Cd budget. The combined model is used to examine the relative contributions of bacteria and dissolved humic substances to Cd complexation in natural settings. Calculations are performed for three representative systems: (1) one with a maximum realistic concentration of bacteria and a minimum realistic concentration of humic substance, (2) one with a maximum realistic concentration of humic substance and a minimum concentration of bacteria, and (3) one with an intermediate concentration of both components.

Our modeling results indicate that dissolved humic substances have 2 orders of magnitude more available binding sites than bacterial surfaces (per gram). Humic substances also have a greater affinity than bacterial surfaces for binding Cd over circumneutral pH ranges. The combined model results demonstrate that, depending upon their relative concentrations, both Cd-humic and Cd-bacteria complexes are capable of dominating Cd-speciation in specific natural environments. This modeling approach is useful in that it can easily be extended to include other metals and binding ligands; however, thermodynamic data must be gathered on additional components to facilitate the modeling of more realistic systems.

1. INTRODUCTION

Bacteria, along with humic (HA) and fulvic (FA) acids, are the main reservoirs for organically-bound metals in most natural waters. As such, they are capable of controlling the availability and cycling of elements and are critical components in the transport of heavy-metal contaminants (Tornabene and Edwards, 1972; Cabaniss and Shuman, 1988; Ma et al., 1999; Tortell et al., 1999). The effectiveness of these substances at binding protons also influences the buffering capacity and chemistry of natural waters. Despite the importance of these complexing agents, direct, quantitative comparisons of the relative binding capacities of bacteria and HA/FA over a wide range of chemical conditions (i.e., ionic strength, pH, metal concentration) have not been attempted. Previous studies that have investigated the relative importance of bacteria and HA/FA have focused only on specific sets of chemical conditions and have been qualitative in nature (Ledin et al., 1996; Ledin et al., 1999). Deciding which of these components is most important for metal binding in a given environment has significant implications for mass transport and remediation solutions. Metals bound to dissolved HA/FA will easily disperse in aquatic environments, whereas the fate of metals bound to bacteria depends upon the size, shape, and mobility of the bacteria. Recent advances in understanding proton and metal binding to both bacterial surfaces (Fein et al., 1997; Cox et al., 1999; Fowle and Fein, 2000; Kelly et al., 2002; Boyanov et al., 2002; Martinez et al., 2002) and HA/FAs (Paxeus and Wedborg, 1985; Ephraim et al., 1986; Benedetti et al., 1996; Christl and Kretzschmar, 2001; Ritchie and Perdue, 2003) for the first time enables these types of quantitative comparisons of the thermodynamic stabilities of HA/FA and bacterial surface complexes.

The best way to quantify the competition for the binding of protons and metals onto bacterial surfaces and HA/FA is through thermodynamic modeling of both components. However, there are significant obstacles to developing a combined model from existing bacteria and HA/FA models. A range of model types, each with their own adjustable parameters, have been used to describe proton and metal binding onto bacteria and HA/FAs. It is impossible to simply combine existing stability constants into a unified thermodynamic model due to these inconsistencies. Existing models generally consist of two parts: (1) description of proton or metal binding onto negatively charged functional group sites, and (2) description of the ionic strength and electric field dependence of the reaction. The description of proton and/or metal binding can be further subdivided into those models that utilize discrete binding sites (Fein et al., 1997; Cox et al., 1999; Pagnanelli et al., 2000, for bacteria; Westall et al., 1995; Tipping and Hurley, 1992; Tipping, 1998; Gustafasson, 2001, for HA/FA) and those that

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utilize a continuous distribution of sites (Plette et al., 1995, 1996, for bacteria; Purdue and Lytle, 1983; Koopal et al., 1994; Milne et al., 1995; Benedetti et al., 1996; Kinniburgh et al., 1996, for HA/FA).

Perhaps the most frequently used continuous site distribution model is the nonideal competitive adsorption (NICA) model (Koopal et al., 1994). The NICA model is based on a bimodal Langmuir isotherm that includes an ion-specific nonideality term and an equilibrium constant width distribution term specific to the humic substance or bacterial species in question. Equilibrium constants, site concentrations, and the nonideality and width distribution terms are empirically-derived by fitting experimental data (Benedetti et al., 1995).

Discrete surface chemical equilibrium modeling has been used successfully to describe the competitive binding of protons and Co onto HA (Westall et al., 1995) and to describe the competitive binding of protons and a variety of metals onto bacterial surfaces (Xue et al., 1988; Fein et al., 1997; Daughney and Fein, 1998; He and Tebo, 1998; Borrok et al., 2003). This modeling approach requires identification of the organic-proton/metal complexes, determination of the thermodynamic stability constants of those complexes, and determination of the concentration of binding sites on the bacterial surface or humic substance.

Most models describe electrostatic interactions between charged species using double-layer theory. HA/FA models differ mainly in how the Poisson-Boltzman equation is solved in accordance with the perceived geometry of the HA/FA molecules (cylindrical or spherical) (Bartschat et al., 1992; Milne et al., 1995; Avena et al., 1999). The size and shape parameters for these electrostatic models are often arbitrarily chosen or are empirically derived to best-fit the data. Constant capacitance electrostatic models have frequently been utilized for bacteria systems (e.g., Fein et al., 1997). The Donnan electrostatic model has been proposed for both HA/FAs and bacteria (e.g., Plette et al., 1995; Benedetti et al., 1996). In this approach, one assumes that all counter ions necessary to balance the surface charge of a HA/FA particle or bacterial surface group are present within a hypothetical sphere with an empirically-derived volume called the Donnan volume (Marinsky and Ephraim, 1986).

Despite the inherent heterogeneity among different HAs and FAs, their overall proton and metal binding affinities vary within fairly narrow limits. These similarities have prompted some researchers to develop “universal” (average) sets of parameters to fit their preferred model. For example, NICA-Donnan parameters for the binding of protons and metals onto dissolved humic substances were recently developed by Milne et al. (2001) and Milne et al. (2003), respectively, for a wide range of HAs and FAs. Similarly, studies have shown that a wide-range of bacterial species adsorb protons and metals to similar extents (Daughney et al., 1998; Small et al., 1999; Yee and Fein, 2001; Kulczycki et al., 2002; Ngwenya et al., 2003; Yee and Fein, 2003), making determination of a “universal” (average) set of discrete equilibrium constants possible. Using this approach, Borrok et al. (2004) developed a set of thermodynamic parameters that describe the adsorption of protons and Cd onto consortia of bacteria cultured from a wide range of natural environments.

In this study, we utilize chemical equilibrium modeling to develop a set of parameters that describe the competitive binding of protons and Cd onto HA/FA. We combine existing modeling parameters developed in a recent bacterial complexation study with the HA/FA Cd complexation results from this study to form a unified, internally consistent, HA/FA/bacteria Cd complexation model. We use the unified model to explore the relative importance of bacteria and HA/FA in binding Cd under realistic conditions. This study is not designed to critique or debase existing models that describe the complexation of metals onto bacterial surfaces or HA/FAs. Clearly, as described above, a number of existing models are capable of describing the same data sets utilized here. Instead, we introduce this model to facilitate the direct quantitative comparison of the adsorptive capacities of bacteria and HA/FA. This model is advantageous because it utilizes the same formalism as many existing contaminant transport codes, while effectively describing proton and Cd binding to bacteria and HA/FA over a range of chemical conditions.

2. CHEMICAL EQUILIBRIUM MODELING

2.1. Basis of the Chemical Equilibrium Model

The chemical equilibrium modeling approach explicitly accounts for the complexation of protons and metals with the ligand of interest, using experimental data to solve for equilibrium constants and/or concentrations of individual chemical species in a system (e.g., Westall et al., 1995; Fein et al., 1997). We represent functional groups (present either on bacterial surfaces or on dissolved humic substances) using a number of discrete monoprotic acids, each of which undergoes the following deprotonation reaction:

$$\text{R-AH}^\circ \Leftrightarrow \text{R-A}^- + \text{H}^+ \quad (1)$$

where R is the bacterium or humic substance to which the functional group type, A, is attached. The acidity constant, K_a, for reaction (1) can be expressed as:

$$K_a = \frac{[\text{R-A}^-]a_{\text{H}^+}}{[\text{R-AH}^\circ]} \quad (2)$$

where $[\text{R-A}^-]$ and $[\text{R-AH}^\circ]$ represent the concentration of deprotonated and protonated sites, respectively, and a_H^+ represents the activity of protons in the bulk solution. Metal complexation with the deprotonated ($x = 0$) or protonated ($x = 1$) forms of the monoprotic acids can be expressed as:

$$\text{M}^{m+} + \text{R-AH}_x^{(x-1)} \Leftrightarrow \text{R-AH}_x(\text{M})^{m+(x-1)} \quad (3)$$

where M^{m+} is the aqueous metal cation of interest. The equilibrium constant, K, for this reaction is given by:

$$K_x = \frac{[\text{R-AH}_x(\text{M})^{m+(x-1)}]}{a_{\text{M}^{m+}}[\text{R-AH}_x^{(x-1)}]} \quad (4)$$

where $[\text{R-AH}_x(\text{M})^{m+(x-1)}]$ is the concentration of the metal-ligand complex of interest, a_M^{m+} is the aqueous activity of the metal cation, and K_0 and K_1 refer to the equilibrium constants for Cd binding onto deprotonated and protonated sites, respectively.

We utilize the program FITEQL 2.0 to solve for functional group site concentrations, and proton and metal binding con-

Table 1. Functional group site concentrations (moles per liter per wet gram of bacteria) and proton and Cd binding constants, with corresponding 1 σ uncertainties, for bacterial consortia model.

Bacteria model	Site 1	Site 2	Site 3	Site 4
Proton binding constants (pK_a)	3.12 ± 0.13	4.70 ± 0.11	6.57 ± 0.17	8.99 ± 0.21
Site concentrations	6.65E-5 ± 1.9E-5	6.78E-5 ± 2.7E-5	3.68E-5 ± 1.7E-5	4.47E-5 ± 2.2E-5
Cd binding constants (K)	2.83 ± 0.30	2.70 ± 0.47	3.95 ± 0.22	5.22 ± 0.40

stants (Westall, 1982). The relative goodness of fit of each tested model is quantified using the residual function, V(Y), from the FITEQL 2.0 output for each model. A V(Y) value of 1 signifies a perfect fit, while V(Y) values between 0 and 20 can be considered good fits (Westall, 1982).

2.2. Existing Bacteria Model

Borrok et al. (2004) developed a single set of functional group site concentrations and equilibrium constants that are able to describe the competitive adsorption of protons and Cd onto consortia of bacteria cultured from a range of soil and aquatic environments. We use this bacterial complexation model as a template for the HA/FA modeling discussed below. In the Borrok et al. (2004) study, a nonelectrostatic model was used because proton and Cd binding experiments were performed only at one ionic strength (0.1 m), making it difficult to independently verify the need for an electrostatic correction.

We first determined the minimum number of discrete functional group types that were required to account for the observed buffering capacity of each bacterial consortium by sequentially testing models with one through five proton-active sites. The best-fit models for all the bacterial consortia proton-binding data sets involved the binding of protons onto 4 discrete functional group sites. In each case, five site models were underconstrained and failed to converge. The average pK_a values (3.12, 4.70, 6.57, and 8.99) and functional group site concentrations of the best-fit models are presented in Table 1. We refer to these sites as site 1 through site 4, respectively.

The average pK_a and functional group site concentration values were used to determine cadmium adsorption constants for 10 bacterial consortia Cd-binding data sets. Determination of the best-fit model for these data sets involved testing both monodentate and bidentate Cd complexes with the protonated and deprotonated forms of sites 1 through 4. Models that invoked Cd complexation with the deprotonated forms of each site fit all the data sets best. The weighted average of the Cd binding equilibrium constants for each of the best-fit models (mondodentate binding onto deprotonated sites) are presented in Table 1.

2.3. Modeling Approach for Humic and Fulvic Acids

Our overall modeling approach is similar to that for bacteria (Borrok et al., 2004), and is similar to the approach used by Westall et al. (1995) for Leonardite humic acid. We assume that the acid-base properties of HA and FA can be described by a number of discrete monoprotic acids. As in the Westall et al. (1995) study, we choose a 4-site model and arbitrarily assign pK_a values for each acidic functional group site. Because we want to achieve a direct comparison of binding site densities between bacteria and HA/FA, we use the pK_a values from the bacteria study (3.12, 4.70, 6.57, and 8.99) as the assigned values for the humic system. This approach facilitates the direct comparison of functional group site densities because the concentrations are compared at exactly the same pK_a values. If the pK_a values for each system were different, the direct comparisons we make below would no longer be valid. We model a range of published data sets (Milne et al., 2001), using the chosen pK_a values, and solve for the concentrations of functional group sites that best fit the data. Using the averages of the newly-developed site concentrations and the chosen pK_a values, Cd complexation stoichiometries and their corresponding equilibrium constants are constrained using a range of published Cd binding data sets (Milne et al., 2003).

In addition to this "forced-fit" approach, we also utilize a "best-fit" approach. In the best fit approach, the same proton binding data sets are used to test models that involve 2, 3, 4, or 5 discrete sites, allowing both pK_a values and concentrations of functional group sites to vary to best fit the data. This best-fit approach was adopted for comparison to the forced-fit approach to determine if the arbitrary assignment of pK_a values had a significant effect on the model fits for the proton-binding data sets.

Both the forced-fit and best-fit approaches are simplifications of the molecular-scale speciation of the system. HA/FAs are rich with carboxylic and phenolic functional groups that likely deprotonate over a continuous pH range. Hence, each monoprotic acid in our model does not represent a separate binding ligand with a unique identity, but instead represents the average of a number of similar ligands over a limited pH range. However, the discrete-site approach is powerful in that it simplifies an extremely complex system where a true mechanistic representation of the speciation may be unattainable. As with the bacteria model, electrostatic effects are not explicitly accounted for in our HA/FA models because we focus solely on one ionic strength value (I = 0.1 m). Furthermore, without external well-defined size and shape parameters, the explicit representation of an electrostatic term is difficult to justify (Avena et al., 1999).

3. DATA SELECTION AND MODEL INPUT

3.1. Proton Binding Data

The proton binding data sets used in this study were chosen from the forty-nine individual published and unpublished data sets compiled by Milne et al. (2001). Nineteen of these data sets were previously collected and used for calibration of Model VI (Tipping, 1998); eighteen of these data sets were also utilized in the development of the Stockholm Humic Model (Gustafsson, 2001). The HA/FAs involved in the experiments that are

included in the data compilation were isolated from a range of soil and aquatic environments, and thus represent a broad spectrum of dissolved natural organic matter.

Although the compilation of Milne et al. (2001) consists of 49 data sets, we used only a subset of these. Individual data sets were chosen for use in our modeling according to specific criteria that best facilitated comparison to the existing bacterial consortia model. Initially, only data sets with an ionic strength of 0.08 to 0.12 m (approximately the same ionic strength used in acid-base titrations of the bacterial consortia) were chosen. After development of the comparative model, additional data sets with varying ionic strengths (0.01 to 0.3 m) were chosen (according to the same following criteria) to determine the ionic strength dependence of our calculated results and to examine the usefulness of an explicit electrostatics term in the protonation model. Only data sets that included the pH range 3.6 to 9.5 were chosen, because this was closest to the pH range covered in the acid-base titrations of the bacterial consortia (pH 2.7–9.7). Most of the HA/FA potentiometric titration experiments that are included in the Milne et al. (2001) data set were not conducted to pH values significantly below pH 3.6. For the majority of humic substances included in the database, only one data set was available that met the above criteria. For some humic substances, however, multiple data sets were available. Therefore, to avoid weighting the results toward a specific HA or FA, only one potentiometric titration was used in these cases. Finally, we used only those data sets for which an experimental concentration of HA/FA was reported. In several instances, the starting concentration of HA/FA could be determined by revisiting the published source of a data set, so those data sets were included in our treatment. In summary, 9 FA and 8 HA potentiometric titration data sets from the Milne et al. (2001) compilation met the criteria described above. We retain the numbering convention of Milne et al. (2003) for these data sets for ease of reference.

Each data set that we used included the following parameters: Q (total charge on HA/FA in equivalents/kg), [H+], pH, ionic strength, and [HA/FA], where brackets denote concentration. We were able to use this information to solve for the concentration of acid added minus the concentration of base added [Ca-Cb] for each point in the titration according to the following charge balance equation:

$$[\mathrm{Ca} - \mathrm{Cb}] = [-\mathrm{Q}] + [\mathrm{H}^+] - [\mathrm{OH}^-] \quad (5)$$

We use [Ca-Cb], pH, and [HA/FA] as serial data for input into the chemical equilibrium program FITEQL 2.0, and use the program to solve for site concentrations (and proton binding constants in the best-fit model). Although the charge balance equation holds for every point along the titration, it does not account for the initial charge (Q°) on the humic substance before beginning the titration. Q° represents the concentration of protons it would take to balance the initial charge deficiency at the start of the titration. The Q° term has no effect on the shape of the titration curve, but does affect its relative position (Avena et al., 1999). Hence, we treat Q° as an adjustable parameter and solve for it using FITEQL (see Westall et al., 1995, for a complete discussion).

3.2. Metal Binding Data

We chose seven Cd binding data sets to use in this study from the 171 individual published and unpublished metal binding data sets compiled by Milne et al. (2003) for HA/FAs. The experiments represented in the Milne et al. (2003) compilation involved a range of metal cations as well as a range of HA and FA isolated from both aquatic and soil environments. Because the only metal binding constants that we determined for bacterial consortia are those for Cd (Borrok et al., 2004), in this study we only considered data sets from the Milne et al. (2003) compilation that involved Cd binding. In addition, measurements of Cd binding as a function of pH are critical to fully constrain the stoichiometry of the important Cd complexes. Therefore, only data sets from the Milne et al. (2003) compilation that included Cd binding measurements from at least 3 pH conditions were considered. KNO_3 or $NaNO_3$ were typically used to control the ionic strength in the Cd binding experiments. Only data sets with ionic strength values from 0.01 to 0.2 m were utilized. The ionic strength requirement for the metal binding data was made less stringent, because the number of possible data sets was limited. Finally, as was the case for the proton binding data, we used only those data sets for which an experimental concentration of HA/FA was reported. Of the 171 data sets included in the Milne et al. (2003) compilation, 5 FA and 2 HA data sets met the described criteria. We also used an additional data set (from Liu and Gonzalez, 2000), which was not included in the compilation of Milne et al. (2003) but which met all the above criteria, bringing the HA total to 3 data sets. The numbering convention of Milne et al. (2003) for the data sets is retained for ease of reference.

Each metal binding data set typically includes the following parameters: [Cd bound], [Cd^{+2} free], [H+], pH, ionic strength, and [HA/FA], where brackets denote the concentration of a species. Knowing [Cd^{+2} free], [Cd bound], and solving for [$CdNO_3^+$] and [$Cd(NO_3)_2$], using the equilibrium constants from Smith and Martell (1987), we were able to calculate total Cd (T_{Cd}) at every experimental point. We use T_{Cd}, pH, and [Cd bound] as serial data for input for FITEQL 2.0 and solve for Cd complexation constants. Cd hydrolysis products, including $Cd(OH)^+$, $Cd(OH)_2^\circ$, $Cd(OH)_3^-$, $Cd(OH)_4^{2-}$, $Cd_2(OH)^{3+}$, $Cd_4(OH)_4^{4+}$, were also considered in the speciation calculations with equilibrium constants taken from Baes and Mesmer (1976). Because the concentration of HA/FA often varied slightly due to dilution effects during the course of the experiments, the concentrations of the individual functional group sites were also entered as serial data where appropriate.

4. RESULTS AND DISCUSSION

4.1. Proton Binding to HA/FA

The 4-site models with fixed pK_a values that we invoke to fit the 17 proton binding data sets yield excellent fits to the experimental data over the entire pH range of the measurements. A representative model fit typical of those found for all the data sets is presented in Figure 1. The calculated site concentrations for each of the four sites considered are presented in Table 2, along with corresponding V(Y) values for each model fit. Despite the diversity of the origins of the

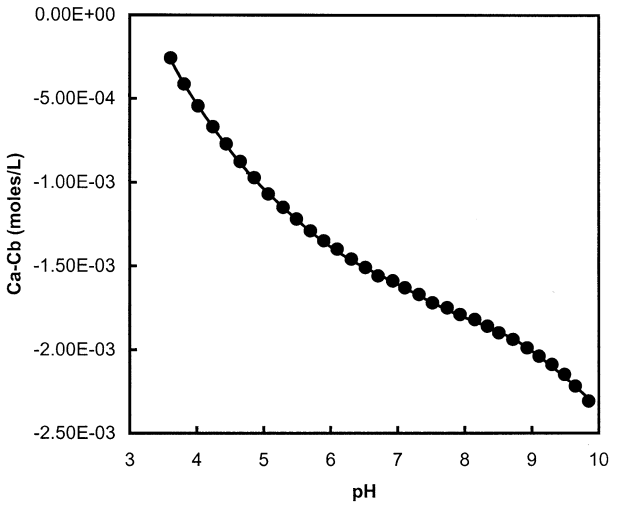

Fig. 1. Potentiometric titration data (solid circles), and best-fit model (curve), for humic acid sample HH-8. Values are positive for net acid added; negative for net base added.

samples and the inherent heterogeneity of HA/FAs, the site concentration numbers for most of the data sets are similar. There are several outliers that contribute to relatively high standard deviations in the data (Table 2); however, the magnitude of the standard deviations is no greater than those for the bacterial consortia model proposed by Borrok et al. (2004).

Direct comparison of the functional group concentrations for sites 1 through 4 for the bacteria and HA/FA models reveals that HA/FAs have ~2 orders of magnitude more sites per gram than do bacteria. This is not surprising because only the outer surfaces of bacteria (mainly the peptidoglycan layer) contain the functional group sites capable of complexing cations (Beveridge, 1989), while dissolved HA/FA particles contain functional groups throughout their structures (Thurman, 1985). HA/FAs have similar functional group site densities compared to dried peptidoglycan on a per gram basis. This comparison illustrates the high density of binding sites on HA/FAs.

For the best-fit modeling approach, we determine the minimum number of discrete functional group types that are required to account for the observed buffering capacity of each HA/FA by sequentially testing models with two through five proton-active sites. In each case, 4-site models yield the best fits to the experimental data over the entire pH range of the measurements. Five-site models in each case do not converge, indicating that that the models are under-constrained and that the data do not support a model with 5 discrete functional group types. This result supports the choice of four discrete sites for the forced-fit model. Additionally, the best-fit models did not fit the data sets any better than the forced-fit models. In fact, the average V(Y) value for the forced-fit models (1.03) is slightly closer to a perfect fit of 1.0 than the average V(Y) from the best-fit models (0.37). The average best-fit pK_a values of 3.99, 5.39, 7.17, and 9.21 (data not shown) are all shifted to slightly higher pH than the forced-fit pK_a values. The reason for this upward shift in pH is that the best-fit models are strongly influenced by the pH ranges of the data sets. Most HA/FA data sets do not involve measurements significantly below pH 3.5 because of potential precipitation problems involving the HA or FA, but generally contain data to high pH (>10). Conversely, titrations using bacteria are often performed (and show buffering capacity) to a pH of 2.5, but are not performed to high pH conditions because cell lysis becomes rapid and significant above approximately pH 9.5 to 10.0.

Discrete sites account for buffering capacity over a more narrow pH range than does a continuous distribution of sites. Therefore, a relatively high number of discrete sites are neces-

Table 2. Best-fit functional group site concentrations (moles per liter per gram of humic substance), with V(Y) values, for each of the four fixed pK_a sites.[a]

HA/FA Sample ID	Material	V(Y)	Site 1 (pK_a 3.12)	Site 2 (pK_a 4.70)	Site 3 (pK_a 6.57)	Site 4 (pK_a 8.99)
FH5	Gota River	0.91	2.24E-03	2.19E-03	8.06E-04	4.08E-04
FH6	Satilla River	0.82	1.47E-03	1.54E-03	7.37E-04	7.38E-04
FH7	Gota River	0.68	1.68E-03	1.28E-03	5.70E-04	6.42E-04
FH10	Whitray Beck	0.51	2.69E-03	1.64E-03	1.00E-03	1.02E-03
FH16	Tuse	0.55	2.48E-03	2.22E-03	6.27E-04	8.00E-04
FH20	Derwent	0.72	2.09E-03	1.90E-03	7.56E-04	7.10E-04
FH21	Kranichsee	0.63	1.59E-03	1.53E-03	8.13E-04	8.58E-04
FH23	Laurentian Soil	1.10	1.89E-03	1.85E-03	1.50E-03	1.81E-03
FH25	Strichen FA	0.77	1.77E-03	2.09E-03	8.07E-04	7.41E-04
HH8	Whitray Beck	0.85	9.06E-04	1.48E-03	9.11E-04	9.89E-04
HH9	PPHA	3.67	2.83E-04	1.29E-03	8.41E-04	1.04E-03
HH11	Eliot Silt Loam	1.16	1.55E-03	1.87E-03	9.67E-04	1.34E-03
HH12	Kranichsee HA	0.92	1.11E-03	1.29E-03	7.26E-04	9.24E-04
HH20	Vejen Landfill	1.13	1.31E-03	1.41E-03	7.67E-04	1.12E-03
HH21	Vejen Landfill	1.40	7.40E-04	8.42E-04	6.07E-04	2.04E-03
HH22	Tongbersven Forest	1.10	6.85E-04	9.91E-04	6.19E-04	6.66E-04
HH24	Aldrich HA	0.61	6.22E-04	2.11E-03	9.27E-04	7.84E-04
Average		1.03	1.48E-03	1.62E-03	8.23E-04	9.78E-04
Std. Dev.		0.72	6.88E-04	4.15E-04	2.16E-04	4.17E-04

[a] Average values and corresponding 1 σ uncertainties are presented at the base of the table. Sample material and identification numbers are from Milne et al. (2001). The data sets used for modeling were collected at an ionic strength range of 0.08 to 0.12 m.

Table 3. Best-fit functional group site concentrations (moles per liter per gram of humic substance) for the ionic strength range of 0.01 to 0.3 m, with V(Y) values for each of the four fixed pK_a sites.[a]

HA/FA Sample ID	Material	Ionic Strength	V(Y)	Site 1 (pK_a 3.12)	Site 2 (pK_a 4.70)	Site 3 (pK_a 6.57)	Site 4 (pK_a 8.99)
FH16	Tuse	0.010	6.25	1.79E-03	2.42E-03	1.03E-03	1.50E-03
FH20	Derwent	0.030	1.83	1.69E-03	1.88E-03	7.71E-04	1.01E-03
FH21	Kranichsee	0.300	6.11	1.71E-03	1.49E-03	5.90E-04	1.72E-03
FH23	Laurentian Soil	0.010	4.73	1.21E-03	1.96E-03	1.44E-03	4.32E-03
FH25	Strichen FA	0.012	4.31	1.19E-03	2.27E-03	9.40E-04	1.11E-03
HH9	PPHA	0.300	2.71	5.02E-04	1.23E-03	7.32E-04	1.37E-03
HH11	Eliot Silt Loam	0.020	14.81	9.97E-04	1.87E-03	8.80E-04	4.56E-03
HH12	Kranichsee HA	0.010	2.75	9.36E-04	1.88E-03	1.00E-03	1.28E-03
HH20	Vejen Landfill	0.010	3.88	9.84E-04	1.42E-03	8.86E-04	1.65E-03
HH21	Vejen Landfill	0.035	7.18	6.17E-04	8.59E-04	5.82E-04	2.80E-03
HH22	Tongbersven Forest	0.300	4.28	7.97E-04	9.90E-04	4.92E-04	1.14E-03
HH24	Aldrich HA	0.010	20.17	6.20E-04	1.64E-03	1.39E-03	1.32E-03
Average		NA	6.58	1.09E-03	1.66E-03	8.95E-04	1.98E-03
Std. Dev.		NA	5.45	4.45E-04	4.81E-04	2.97E-04	1.24E-03

[a] Average values and corresponding 1 σ uncertainties are presented at the base of the table. Sample material and identification numbers are from Milne et al. (2001). NA = not applicable.

sary to describe the observed continuous buffering capacity of HA/FAs and bacteria over the pH ranges used in this study. The best fit model for HA/FA and the previously-developed best-fit model for bacterial consortia demonstrate that 4 discrete sites are needed to account for the continuous buffering capacity that was observed over a pH range of approximately 3 to 9.5. As demonstrated above, the forced-fit and the best-fit approaches both can adequately account for the observed buffering behavior, suggesting that while the potentiometric titration data may constrain the number of discrete sites that are required to account for the buffering capacity, the data do not uniquely constrain the pK_a values and site concentrations. The forced-fit model, although not unique, facilitates comparison of the binding abilities of bacteria and HA/FA molecules. Clearly, a true mechanistic model of the protonation of the bacteria and HA/FA molecules would be more complex, and would require a molecular-scale understanding of the important proton binding reactions. We propose our simplified modeling approach as a means for estimating proton and metal speciations in bacteria- and HA/FA-bearing systems until such a comprehensive mechanistic understanding of these reactions is obtained.

4.2. Electrostatic Interactions

In an effort to determine the magnitude of electrostatic effects on proton binding in this study, twelve additional proton binding data sets (5 FA and 7 HA) with ionic strengths ranging from ~0.01 to 0.3 m were modeled using the forced-fit approach for comparison to the 0.1 m ionic strength modeling results. As before, these data sets were modeled without using any electrostatic corrections. The functional group site concentrations from the models of the variable ionic strength data sets are presented in Table 3, along with their corresponding V(Y) values. The V(Y) values for the variable ionic strength models are generally larger than those found for the 0.1 m ionic strength models. This disparity in the model fits is the result of slight variations in the positioning of the titration data due to electrostatic effects. However, with the possible exception of data set HH-24, with an ionic strength of 0.01 m, the variable ionic strength models still fit the data well. The modeling results indicate that the magnitude of the electrostatic effects (upon calculated functional group site concentrations) is small in comparison to the magnitude of these differences caused by the inherent heterogeneity of the bulk system. In other words, the uncertainties inherent in dealing with multiple HA/FAs isolated from a range of environments outweighs the uncertainties encountered by changing the ionic strength over the ranges tested here. For example, the average site concentrations from the variable ionic strength models are nearly identical to the averages from the 0.1 m ionic strength models for the sites with pK_a values of 3.12, 4.70, and 6.57 (Tables 2 and 3). The average site concentrations for the variable and 0.1 m ionic strength models are somewhat different for the 8.99 pK_a site. However, the differences still fall within the standard deviations measured.

Hence, it is difficult to argue that an electrostatic correction (over the ionic strength range 0.01 to 0.3 m) is warranted for proton binding when dealing with the overall behavior of HA/FA over a range of natural environments. Electrostatics may need to be considered for specific systems where proton binding behavior must be described more precisely than the uncertainties reported here.

4.3. Cd Binding to HA/FA

Model fits of the 8 Cd binding data sets (using the average HA/FA site concentrations developed earlier) were tested for monodentate and bidentate binding onto the protonated and deprotonated forms of discrete sites 1–4 on the humic substances. The model fits for Cd binding are generally very good (Fig. 2), and are characterized by monodentate Cd complexation with deprotonated sites. However, in some cases the complexation mechanisms are difficult to constrain because the experimental data were collected over too narrow a pH range. Furthermore, Cd complexation constants could only be constrained for the functional group sites that were active over the pH range of the data. In the cases where a clear binding stoichiometry could not be identified, monodentate Cd com-

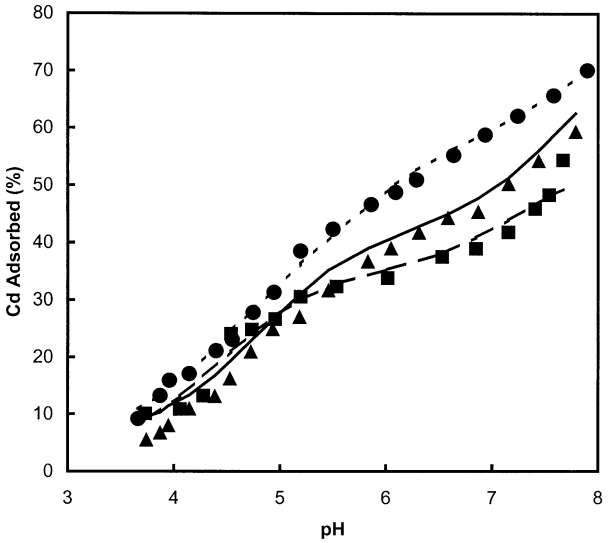

Fig. 2. Cd binding data (solid shapes), and best-fit models (curves), for fulvic acid data FHCd4a, FHCd4b, and FHCd4c. Solid circles = FHCd4a, 22 ppm Cd and ionic strength = 0.01 m; solid squares = FHCd4b, 45 ppm Cd and ionic strength = 0.01 m; triangles = FHCd4c, 24 ppm Cd and ionic strength = 0.1 m.

plexation with deprotonated sites was assumed. The calculated equilibrium constants, for Cd complexation with each deprotonated functional group site, are presented in Table 4. The standard deviations of the equilibrium constants are of similar magnitude to those previously developed for the bacterial consortia model (Table 1). However, the equilibrium constant of 3.68 (data from Liu and Gonzalez, 2000) for site 3 appears to be an outlier, which greatly magnifies the standard deviation of the data for this site. Direct comparison of the stability constant values for Cd-bacteria and Cd-HA/FA complexes reveals that constants for sites 1 and 4 are nearly identical for bacteria and HA/FA, while sites 2 and 3 on the HA/FA have greater affinities for binding Cd than do sites 2 and 3 on the bacteria.

4.4. Competition for Protons and Cd in Natural Waters

Direct comparisons of the magnitude of bacterial and humic substance proton and Cd binding constants are useful in that they provide insights into the relative importance of each component on a per gram basis. However, this is not a true indication of how the components will interact with protons and Cd in natural systems because the concentration of each component can vary greatly from system to system. In addition, each component does not act individually, but competes with the other (and other ligands) for the binding of protons and metals. To examine this competition, we developed a joint model that uses the average parameters given in Tables 2 and 4 to explicitly account for the competition between bacteria and HA/FA for protons and dissolved Cd. In this joint model, we assume that ternary complexation reactions between Cd, bacteria, and HA/FA can be neglected, and that potential binding between bacteria and HA/FA does not significantly alter functional group site concentrations. Preliminary indications suggest that these assumptions are valid for the bacteria and HA/FA concentrations considered here (Wightman and Fein, 2001).

This joint model enables us to assess for the first time the relative importance of bacteria and HA/FA binding on the overall speciation of a heavy metal such as Cd. The model is not meant to be an accurate representation of Cd speciation in natural environments, because a variety of other binding agents (e.g., mineral surfaces, bacterial extracellular polysaccharides, etc.) that are not considered in the model may be present. Hence, the absolute concentrations of bacteria-Cd and HA/FA-Cd complexes in this model are meaningless because other surfaces or ligands could be important or even dominant in natural settings. It is the relative contributions of bacterial cell walls and HA/FA to binding Cd that are determined with the joint model. The internally consistent framework of the model provides a template for the future expansion to other metals and binding components.

We use the combined bacteria/HA/FA model to determine Cd speciation at, and between, the extreme end members of realistic bacterial and HA/FA concentrations expected in natural settings. Many studies exist that explicitly measure the concentrations of dissolved humic substances in natural settings (see Thurman, 1985, for a summary). These values range from very low (~0.05 mgC/L) in some seawater and groundwater systems to greater than 30 mgC/L in some "colored water" systems, including wetlands, swamps, and bogs. Total

Table 4. Cd-HA/FA complexation constants for best-fit Cd binding models (monodentate complexes onto deprotonated sites). Average Cd complexation constants and their corresponding 1 σ uncertainties are presented at the base of the table.[a]

HA/FA ID	Material	Cd (ppm)	Ionic Strength	Site 1 (pK_a 3.12)	Site 2 (pK_a 4.70)	Site 3 (pK_a 6.57)	Site 4 (pK_a 8.99)
FHCd1	Oyster River	5–30	0.1	3.44	3.44	4.87	NA
FHCd2	Podzol Soil	5–30	0.1	3.01	4.12	5.26	NA
FHCd4a	Laurentide	22	0.01	2.87	3.75	4.8	5.35
FHCd4b	Laurentide	45	0.01	2.64	3.31	5.7	5.49
FHCd4c	Laurentide	24	0.1	2.71	NA	5.9	5.6
HHCd3	PPHA	9–60	0.1	NA	3.7	4.76	NA
HHCd6	Okchun Soil	3–15	0.1	NA	3.94	4.63	NA
HHCd	PPHA	10	0.2	2.49	3.08	3.68	NA
Average				2.86	3.62	4.95	5.48
Std. Dev.				0.34	0.36	0.69	0.13

[a] Sample material and identification numbers are from Milne et al. (2001) except for HHCd (Liu and Gonzalez, 2000). Data set FHCd4 has been broken into 3 parts (a, b, c). The data sets used for modeling were collected at an ionic strength range of 0.01 to 0.2 m. NA = not applicable for best model fit.

bacteria counts have also been measured in a variety of natural settings. Reported values range from $\sim 1 \times 10^4$ to 1×10^7 cells/mL in fresh surface waters (Fisher et al., 1998; Almeida et al., 2001; Kisland et al., 2001; Young, 2003). Larger concentrations (up to 1×10^8 cells/g) of bacteria have been reported for aquifer and lake sediments (Balkwill and Ghiorse, 1985; Zeng and Kellogg, 1994; Barns and Nierzwicki-Bauer, 1997; Alfreider et al., 1997; Martino et al., 1998). Concentrations as high as 1×10^9 cells/g have been reported for organic-rich soils (Barns and Nierzwicki-Bauer, 1997). Bacterial concentrations in seawater (5×10^5 to 3×10^6 cells/mL) are generally intermediate in value (Zweifel and Hagstrom, 1995).

The first end member calculation uses a low value of 0.05 mgC/L for HA/FA and a near-maximum value of 1×10^8 cells/mL for bacteria. The situation is then reversed in the second end member calculation and we use a high concentration of HA/FA (20 mgC/L) and set the concentration of bacteria to a low value (1×10^4 cells/mL). We also model Cd speciation in a hypothetical "intermediate" situation where the concentrations of bacteria (1×10^7 cells/mL) and HA/FA (1 mgC/L) are more typical of what might be expected in some natural environments. The concentrations of HA/FA and bacteria used in these calculations are not meant to be indicative of specific environments, but were chosen to represent possible concentrations in natural systems. Bacteria concentrations were converted from cell counts to wet weight based on the assumptions that the average size and shape of the bacteria in each environment is similar to *Escherichia coli*, and that the average cell density is 1.04 g/cm^3. Although there is a large degree of uncertainty associated with these assumptions, small changes in bacterial size and density have only a minor effect on the model results. We use the joint model to calculate the end member distributions of Cd for a total Cd concentration of 50 ppb over the pH range 2–10.

The results of the end member predictive models are presented in Figures 3 and 4, and the results of the intermediate model are presented in Figure 5. The results show that Cd-bacteria complexes dominate over Cd-HA/FA complexes in the first end member situation (Fig. 3), and that Cd-HA/FA complexes dominate over Cd-bacteria complexes in the other end member scenario (Fig. 4). Cd-bacteria and Cd-HA/FA complexes may both need to be considered in environments where the concentrations of these components lie between the end member concentrations (Fig. 5), although these complexes represent only a small fraction of the total Cd in the system. At high (end member) concentrations, both Cd-bacteria and Cd-HA/FA complexes represent a significant fraction of the total Cd in the model systems. In other words, depending upon their respective concentrations, bacteria and HA/FA both have the capability to significantly affect Cd speciation in natural environments. At the total Cd concentration used in the modeling, Cd-HA/FA complexes appear to be significant over most of the concentration ranges of HA/FA that might be expected in natural systems, while Cd-bacteria complexes are relatively insignificant when bacteria concentrations dip below approximately 1×10^6 cells/mL. However, because these models do not include other competing cations (e.g., Ca, Mg, Cu, Fe, Al) or other binding surfaces or ligands that may be present, the concentrations reported here are likely maximum values.

In addition, the ratios of organic components to metal concentrations examined using this joint surface complexation model are similar to the component/metal ratios used to develop the modeling parameters. It is assumed that this modeling approach can be extended to include a wide range of organic/metal concentration ratios; however, the dominant adsorption mechanism on which these modeling parameters are based may be different under extremely high or low metal loading conditions. If so, the equilibrium constants for these additional reactions must be determined to model the effect of bacterial and HA/FA complexation on metal speciation in these systems.

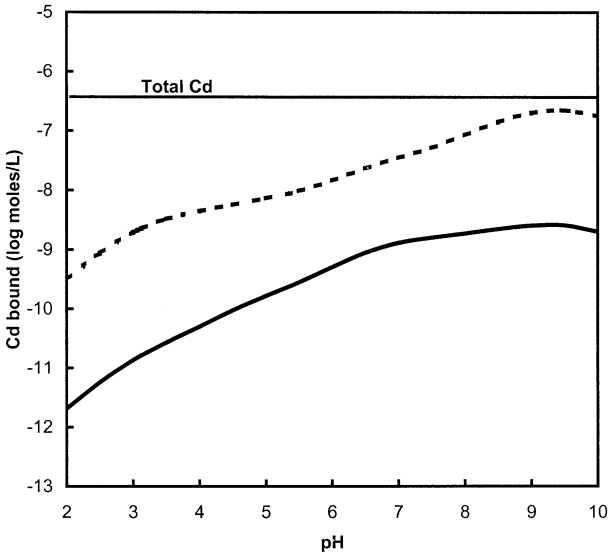

Fig. 3. Predictive model results for end member 1 scenario (0.05 mgC/L HA/FA, 1×10^8 cells/mL bacteria, and 50 ppb Cd). Solid curve represents Cd-HA/FA complexes and dashed curve represents Cd-bacteria complexes.

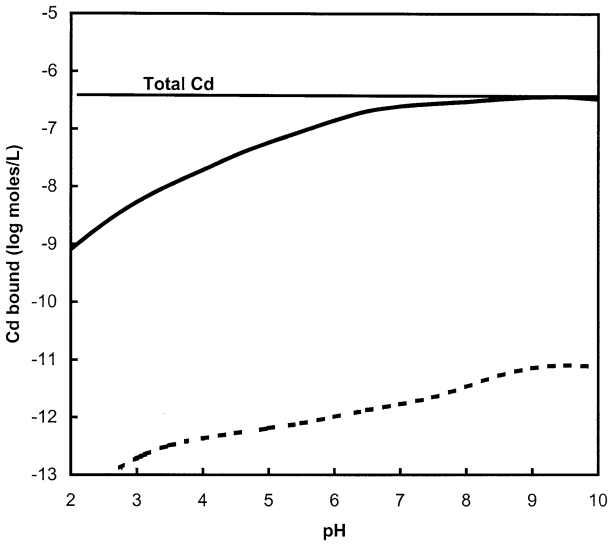

Fig. 4. Predictive model results for end member 2 scenario (20 mgC/L HA/FA, 1×10^4 cells/mL bacteria, and 50 ppb Cd). Solid curve represents Cd-HA/FA complexes and dashed curve represents Cd-bacteria complexes.

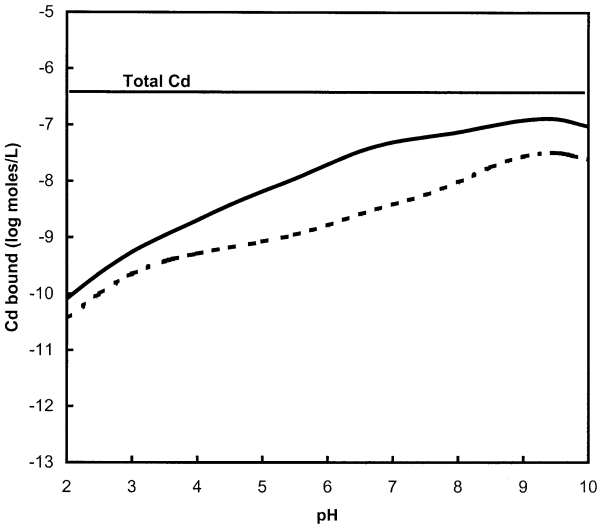

Fig. 5. Predictive model results for an intermediate concentration scenario (2 mgC/L HA/FA, 1×10^7 cells/mL bacteria, and 50 ppb Cd). Solid curve represents Cd-HA/FA complexes and dashed curve represents Cd-bacteria complexes.

5. CONCLUSIONS

In this study, we develop a four-site thermodynamic model that is capable of describing the competitive binding of protons and Cd onto dissolved humic substances (HA/FA). The functional group site densities and complexation constants for this model are internally consistent with the bacterial consortia model previously developed by Borrok et al. (2004), enabling direct comparison of the abilities of the two components to bind Cd. The functional group site densities for HA/FA are approximately 2 orders of magnitude more concentrated than those for bacterial consortia on a per gram basis. Over the circumneutral pH range, Cd-HA/FA complexation constants are approximately one log unit greater than Cd-bacteria complexation constants.

The HA/FA model is combined with the bacterial consortia model to examine Cd complexation over the broad range of possible concentrations in natural systems. Application of the combined model reveals that both bacteria and HA/FA have the capability of significantly affecting Cd speciation in specific natural environments. Development of an internally consistent framework for exploring Cd speciation between bacteria and HA/FA in natural environments represents a large step forward in our ability to describe metal speciation in complex systems. However, this model must be used in conjunction with other binding constants to determine the true speciation of Cd in realistic geologic settings.

This thermodynamic approach involves simplification of a complex chemical system to enable quantitative predictions of the fate of metals in the environment. We are just now in the position of being able to thermodynamically describe some of these complex systems due to recent measurements of thermodynamic stabilities for metal complexes with bacteria and HA/FA. We hope that this study will not only spur on further tests of these predictions, but will spur on further measurements of thermodynamic parameters so that this approach can be extended to include the range of other metals and ligands of environmental and geologic interest.

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