Ternary interactions in a humic acid–Cd–bacteria system

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Abstract

Humic acid adsorption onto the bacterial surface of *Bacillus subtilis* was measured with and without Cd, as a function of pH and humic–bacteria–Cd ratios. These experiments tested for the existence of ternary interactions in a bacteria–humic–metal system. We determine both the effects of humic acid on the bacterial adsorption of Cd, as well as the effects of the aqueous metal cation on the bacterial adsorption of humic acid. The presence of Cd does not affect the extent of humic acid adsorption onto the bacterial surface, indicating that there is no competition for sorption sites between humic acid and Cd under the experimental conditions, and that changes in the charging properties of the bacterial surface, as a result of the Cd adsorption, are not significant enough to affect humic acid adsorption.

The presence of humic acid does diminish Cd adsorption onto the bacterial surface, suggesting the presence of an aqueous Cd–humate complex under mid to high pH conditions. However, we also observe that the solubility of humic acid is unaffected by the presence of aqueous Cd. This apparently inconsistent behavior of an aqueous Cd–humate complex affecting Cd adsorption but not affecting humic acid solubility is not observed with simpler ionizable organic molecules. We propose that the solubility of humic acid is controlled by the solubility of a less soluble fraction of the acid. Cd forms an aqueous complex with the more soluble fraction of humic acid and there is no interdependence between the aqueous activities of the more and less soluble fractions. That is, the solubility of one humic acid fraction is unaffected by the presence of an aqueous Cd–humate complex involving another humic acid fraction. These experimental results constrain the relative importance of surface ternary and aqueous metal–humate complexes on the bacterial adsorption of both humic acid and metal cations. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Ternary interactions; Humic acid–Cd–bacteria system; Bacterial adsorption

1. Introduction

Bacteria can control the fate and transport of contaminants in the subsurface due to their ability to adsorb high concentrations of aqueous metal cations (Beveridge and Murray, 1976, 1980; Harvey and Leckie, 1985; Gonçalves et al., 1987; Fein et al., 1997) and organic molecules (e.g. Baughman and Paris, 1981; Daughney and Fein, 1998a). Bacteria also affect mineral dissolution (e.g. Bennett et al., 1996; Ullman et al., 1996; Grantham et al., 1997; Lee and Fein, 2000) and precipitation (Fortin et al., 1997), and they can degrade a variety of organic contaminants and alter the valence state of redox-sensitive metals through metabolic and/or enzymatic processes (e.g. Lovley et al., 1989, 1991).

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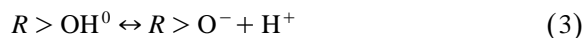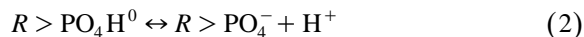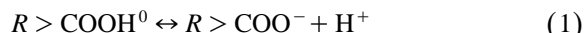*E-mail address:* wightman.1@nd.eud (P.G. Wightman).

Adsorption behavior in binary metal–bacteria, organic–bacteria, and bacteria–mineral systems has been measured experimentally in which Fein and coworkers have developed a surface complexation approach in quantifying the extent of adsorption that occurs in each of these systems (Fein et al., 1997; Daughney et al., 1998; Fowle and Fein, 1999, 2000; Daughney and Fein, 1998b; Yee et al., 2000). However, few studies of ternary systems have been conducted (see Fein, 2001 for a recent review). Of particular interest to modeling contaminant mobility in realistic systems are the ternary interactions between bacterial cell walls, humic acids and heavy metals due to their common occurrence in contaminated sites. Fein et al. (1999) measured humic acid adsorption onto bacterial surfaces, finding extensive adsorption under acidic to near-neutral pH conditions, with decreasing adsorption in increasing pH. This behavior is similar to that observed for humic acid adsorption onto mineral surfaces (Parfitt et al., 1977; Tipping, 1981a,b). The presence of humic acid in humic–metal–mineral ternary systems enhances the adsorption of aqueous metal cations onto positively charged mineral surfaces through the formation of ternary surface–humic–metal complexes under relatively acidic conditions (e.g. Zachara et al., 1994). At higher pH values in these ternary systems (where mineral surfaces become negatively charged and humic adsorption decreases), the presence of humic acid decreases the mineral surface adsorption of aqueous metal cations due to the formation of aqueous humic–metal complexes. Because the presence of humic acids in water–rock systems can affect the extent of metal cation adsorption through the formation of ternary surface– and aqueous metal–humate complexes, it is possible that such ternary interactions also affect metal speciation in bacteria–humic–metal systems as well.

The only means to test for the existence of ternary surface complexes is through direct experimentation. Therefore, in this study, we conduct experiments that test for the existence of ternary interactions in a bacteria–humic–metal system, determining both the effects of humic acid on the bacterial adsorption of an aqueous metal cation, as well as the effects of the metal cation on the bacterial adsorption of humic acid. The experimental results constrain the relative importance of surface ternary and aqueous metal–humate complexes on bacterial adsorption of both humic acid and metal cations, thereby providing a better understanding on the effects of bacteria–metal–humic interactions on contaminant transport in realistic geological systems.

1.1. Bacterial species

The bacterial species used in this study was *Bacillus subtilis*, a gram-positive aerobic species that is commonly found in near surface systems. The cell wall of *B. subtilis* is composed of peptidoglycan and teichoic acids and exhibits carboxyl, phosphoryl, hydroxyl, and amino functional groups at the surface (Beveridge and Murray, 1980). Three types of functional groups (likely carboxyl, phosphoryl, and hydroxyl sites) undergo deprotonation reactions between pH 3.5 and 10.5 and are responsible for an increasingly negatively charged bacterial surface with increasing pH. The deprotonation of the functional groups can be characterized by the site-specific reactions, intrinsic equilibrium constants and site abundances listed below and in Table 1 (from Fein et al., 1997).

$$R > COOH^0 \leftrightarrow R > COO^- + H^+ \qquad (1)$$

$$R > PO_4H^0 \leftrightarrow R > PO_4^- + H^+ \qquad (2)$$

$$R > OH^0 \leftrightarrow R > O^- + H^+ \qquad (3)$$

The effect of the surface potential on the surface speciation is accounted for using the Poisson–Boltzmann charge-distribution equation (e.g. Stumm and Morgan, 1996):

$$K_{intrinsic} = K \exp(-\Delta Z F \psi / RT) \qquad (4)$$

where $K_{intrinsic}$ is the equilibrium constant corrected to zero surface charge. The parameter ΔZ represents the change in the charge of the surface species in the reaction of interest and F, ψ, R and T refer to Faraday's constant, the electric potential of the surface, the gas constant and absolute temperature, respectively. We use a constant capacitance model to relate the electric potential (ψ) to the surface charge (σ), such that:

$$C = \frac{\sigma}{\psi} \qquad (5)$$

where C is the capacitance of the surface layer in F/m^2. This formalization allows us to characterize

the bacterial surface speciation and surface charge as a function of pH (Fig. 1). As Fig. 1 depicts, deprotonated carboxyl groups dominate the surface speciation above pH 4.8 and are responsible for the majority of the surface charge over the entire pH range.

1.2. Humic acid speciation and adsorption

The chemical properties of natural humic substances (elemental composition, aromatic character, functional group composition, ionic character, molecular size and structure) greatly influence both their sorption to surfaces and their aqueous complexation with metal cations. These properties are a function of the source material from which the humic acid is formed. In order to simplify the task of quantifying the specific interactions between humic acids, metals and bacteria, we use only one type of humic acid in this study, supplied by Aldrich Chemical in the form of a sodium salt and isolated from water draining an open pit mine in Oberhessen, Germany (Ochs et al., 1994).

1.2.1. Proton binding to humic acid

The acid–base properties of this humic acid have been characterized by Boily and Fein (2000) using a variety of proton binding models including multicomponent non-electrostatic, multi-component Langmuir–Freudlich and constant capacitance electrostatic models. We discuss our findings in the context of the three-component non-electrostatic model at an

Table 1
Bacteria and humic acid surface models

Equation number[a]	Functional group type	(pK_a)[b,c]	Site concentration[c,d]
Bacteria			
Eq. (1)	Carboxyl	4.82	1.2×10^{-4}
Eq. (2)	Phosphoryl	6.90	4.4×10^{-5}
Eq. (3)	Hydroxyl	9.40	6.2×10^{-5}
Humic acid			
Eq. (6)	L1	2.5	4.46×10^{-3}
Eq. (7)	L2	6.1	2.30×10^{-3}
Eq. (8)	L2	8.8	0.67×10^{-3}

[a] As referred to in text.
[b] Negative logarithm of the intrinsic equilibrium constant for the deprotonation reaction.
[c] Bacteria values from Fein et al. (1997). Humic acid values from Boily and Fein (2000).
[d] Moles of sites per gram.

ionic strength of 0.1 M (based on theory and data presented by Boily and Fein, 2000). The model is characterized by the following reactions:

$$HL1^0 \Leftrightarrow L1^- + H^+ \qquad (6)$$

$$HL2^0 \Leftrightarrow L2^- + H^+ \qquad (7)$$

$$HL3^0 \Leftrightarrow L3^- + H^+ \qquad (8)$$

where HL^0 and L^- represent the protonated and deprotonated form, respectively, of the numbered surface site types. With this approach, the acidity of the molecule is described with distinct acidity constants and site concentrations, as given in Table 1.

The calculated speciation and net charge diagram for the humic acid is shown in Fig. 2. The deprotonation of the first functional group type ($HL1^0$), most likely a consortia of carboxyl groups, dominates the net charge profile of humic acid over a wide range of pH conditions.

1.2.2. Solubility of humic acid

Humic acids are, by traditional definition, low to mid molecular weight organic acids that precipitate below pH 2. In reality, the extent of dissolution varies as a function of pH (Fein et al., 1999), with more humic acid dissolving as the functional groups deprotonate with increasing pH. In this study, each adsorption experiment contains 0.1 g/l of humic acid and we measure the solubility of the experimen-
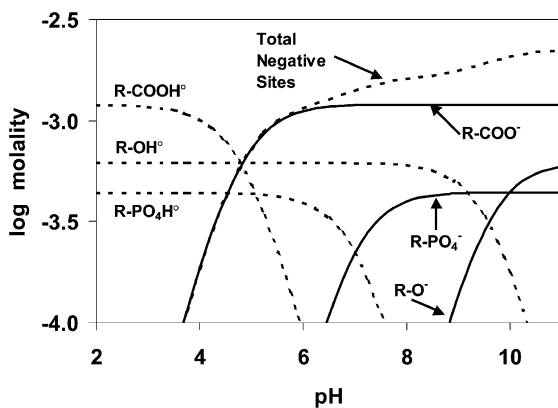

Fig. 1. Speciation diagram for *B. subtilis* based on protonation constants and site abundances from Fein et al. (1997), shown here for a 10 g/l bacteria suspension.
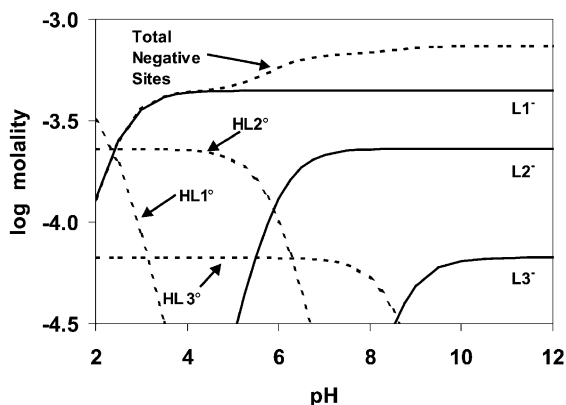
Fig. 2. Speciation diagram for Aldrich humic acid based on protonation constants and site abundances from Boily and Fein (2000). Shown here for a 0.1 g/l suspension. The L1⁻ species dominates the total negative site abundance from low pH to pH 5.

tal humic acid to quantify the amount of humic acid that dissolves as a function of pH. Only by comparing the amount of humic acid in solution in a bacteria- or Cd-bearing system, with that in a humic-only system can we quantify the effects of the other components on humic removal from solution.

1.3. Humic–bacteria adsorption

The adsorption of humic acid onto the bacterial surface of *B. subtilis* has been previously characterized by Fein et al. (1999). The interaction between humic acid and bacteria is mainly hydrophobic and is strongest under low pH conditions wherein both of the bacterial surface and the humic acid molecule are less negatively charged. With increasing pH, the functional groups on both the bacteria and the humic acid deprotonate and become increasingly negatively charged, dramatically inhibiting adsorption at values higher than approximately pH 7. The adsorption behavior can be characterized using the following site specific equilibrium reaction (Fein et al., 1999):

$$HL3^0 + R > COOH^0 \Leftrightarrow R > COOH > HL3^0 \qquad (9)$$
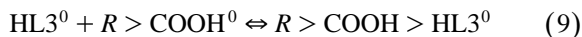
where $HL3^0$ is the functional group on the humic molecule and R represents the bacterial cell wall. The pH dependence of adsorption that is consistent with this adsorption model is shown in Fig. 3 as a dashed curve.

1.4. Metal–humic interactions

The interaction between humic acid and an aqueous metal cation is driven by the electrostatic attraction between the deprotonated functional groups on the humic acid and the positively charged metal cation. The metal binding is likely controlled by the carboxylic and phenolic functional groups (Benedetti et al., 1995), which are more abundant than the other group types (namely amino, sulfhydryl and quinone groups). However, given the complexity and heterogeneity of humic acid molecules, the specific reactions that account for metal uptake by humic acids have not been well-characterized and are likely to vary between humic acid types. In most cases, metal–humic interactions have been characterized using distribution functions and partition models that successfully model the overall interaction (e.g. Benedetti et al., 1995; Koopal et al., 1994) but do not enable extrapolation of systems, such as our ternary systems, that have not been directly studied in the laboratory.

1.5. Metal–bacteria adsorption

The metal chosen for this study is Cd, a heavy metal and E.P.A. priority pollutant (Keith and Tel-
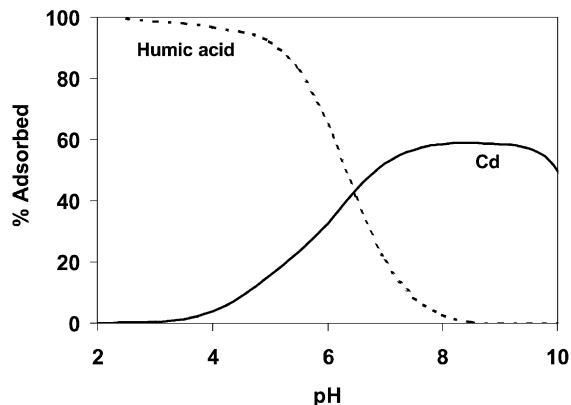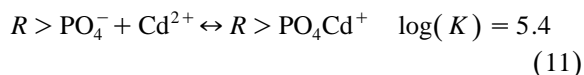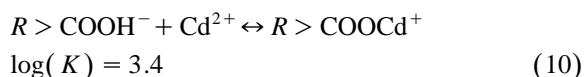

Fig. 3. Adsorption curves for humic acid and Cd onto bacterial surfaces in binary systems, as a function of pH for concentrations approximating those used in our experiments. Humic acid curve is at a concentration of 0.1 g/l humic acid and 2.0 g/l bacteria. Cd curve is at a concentration of 10^{-4} molal Cd and 2 g/l bacteria.

liard,1979). In aqueous solution, Cd exists as a divalent cation throughout the low and mid pH range (Baes and Mesmer, 1976), and a calculated bacterial surface adsorption edge is depicted in Fig. 3. Under low pH conditions, bacterial surface sites are fully protonated and neutrally charged, thus little Cd adsorption occurs. With increasing pH, the surface functional groups become increasingly deprotonated and negatively charged, causing a corresponding increase in Cd adsorption.

Recent studies (Fein et al., 1997; Daughney and Fein, 1998b; Daughney et al., 1998; Fowle and Fein, 1999) have demonstrated that both single- and multi-metal adsorption to bacterial surfaces can be successfully modeled using discrete values for site-specific equilibrium constants in the context of a surface complexation model (SCM). We characterize the metal affinity for the surface of *B. subtilis* using the model and stability constants of Fein et al. (1997), as follows:

$$R > COOH^- + Cd^{2+} \leftrightarrow R > COOCd^+$$
$$\log(K) = 3.4 \tag{10}$$
$$R > PO_4^- + Cd^{2+} \leftrightarrow R > PO_4Cd^+ \quad \log(K) = 5.4 \tag{11}$$

where R represents the bacterial surface to which each functional group type is attached.

2. Materials and methods

B. subtilis cells were obtained from T.J. Beveridge (University of Guelph, Ontario). They were cultured and washed as described by Fein et al. (1997), with the exception of the EDTA treatment, which was limited to 1 h. Growth medium was a 30 g/l trypticase soy broth with 5 g/l yeast extract mixture. Cells were first grown in 5 ml volumes of media for 24 h at 32°C and then were transferred to 3 l volumes of media to grow for another 24 h The wash procedure consisted of two washes in 18 MΩ water purified by reverse osmosis (RO), 1 h exposure to pH 2 nitric acid solution, two washes in RO water, 1 h exposure to 0.001 M EDTA, followed by two washes in RO and two washes in 0.1 M NaClO$_4$. This removed all of the growth media and adsorbed cations from the bacterial surface. Results from our

laboratory indicate that the cells remain viable and are intact following the wash procedure. Humic acid and sodium perchlorate were obtained from Aldrich Chemical and were used without further purification. RO water was used for all growth and experimental solutions.

Experiments involved the reaction of a known amount of Cd, humic acid and bacteria in 15 ml polypropylene test tubes for 2 h. Kinetic experiments (data not shown) indicated that this duration was sufficient to attain equilibrium. We then centrifuged the sample for 3 min at 8000 rpm, measured pH, filtered and analyzed the aqueous fraction. Humic acid concentration was determined by visible light absorption at 450 nm in a dual cell UV–VIS spectrophotometer with 0.1 M NaClO$_4$ in the reference cell. Humic acid standards were filtered at their natural pH of approximately pH 7.5. The presence of Cd in the samples did not affect the humic acid analyses. Analytical uncertainty in the humic concentrations was approximately ± 0.001 g/l. The concentration of Cd in solution was determined by inductively coupled plasma atomic emission spectrometry (ICPAES) using the primary peak at 214.4 nm. All calibration standards were matrix-matched as closely as possible with respect to solution composition, but not corrected for variations in solution pH. Analytical uncertainty in the Cd concentrations is approximately $\pm 4\%$. The amount of humic and Cd adsorbed onto the bacteria was determined by the difference between the known initial total concentration and the concentration in the filtered aqueous fraction after equilibration. For the purpose of this study, we define the aqueous fraction as anything not filtered by a 0.45-μm nylon syringe filter. *B. subtilis* cells have dimensions of 5×1 μm and are adequately filtered out of suspension using these types of filters, as confirmed by visual inspection.

Four types of experiments were undertaken at a constant ionic strength of 0.1 M sodium perchlorate: (1) solubility measurements of humic acid alone and in the presence of Cd as a function of pH; (2) adsorption measurements of humic acid onto the bacteria with 0.1 g/l humic acid and either 0.5 or 2.0 g/l bacteria, conducted as a function of pH; (3) experiments designed to determine the effect of Cd on humic acid adsorption to bacterial surfaces, using systems with proportionately high concentrations of

aqueous Cd (we measured humic acid and Cd adsorption as a function of pH at a constant humic acid concentration of 0.1 g/l, with Cd concentrations of either 10^{-4} or $10^{-3.5}$ molal and with two bacterial concentrations (0.5 and 2 g/l)); and (4) experiments designed to determine the effect of humic acid on Cd adsorption to bacterial surfaces, using systems with proportionately high concentrations of adsorbed humic acid (we measured Cd and humic acid adsorption as a function of humic acid concentration at constant pH, with $10^{-4.05}$ molal Cd in a 10 g/l bacteria suspension).

3. Results

3.1. Humic–Cd experiments

The addition of Cd to humic acid solutions does not cause a significant change in the solubility in solutions of low to circumneutral pH (Fig. 4). Experiments conducted at 10^{-4} and $10^{-3.5}$ molal Cd and 0.1 g/l humic acid have statistically equivalent trends from pH 1.7 to 7.0. At pH values greater than 7, the 10^{-4} molal Cd solution exhibits identical solubility to that of the humic acid alone and both exhibit virtually complete dissolution of the humic acid in the experimental system. However, above pH 7, the
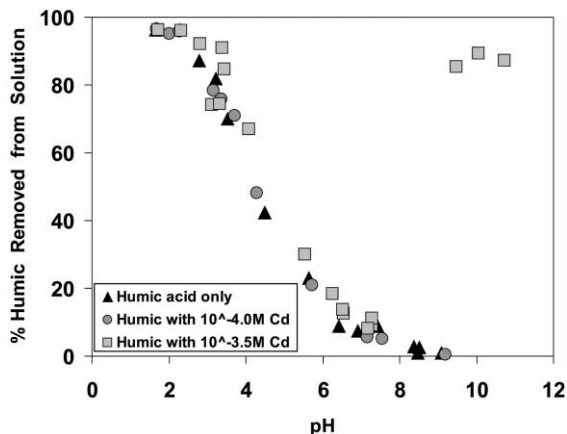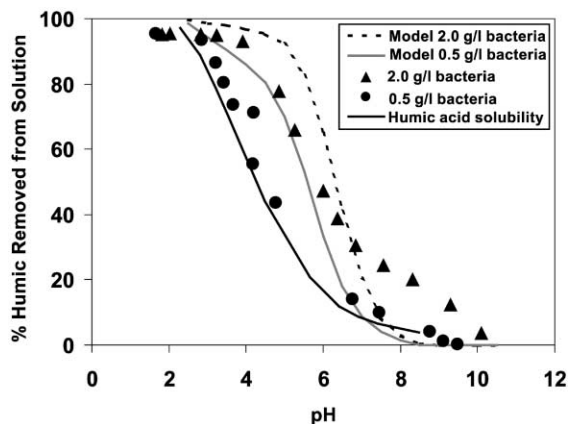

Fig. 5. Humic acid adsorption onto *B. subtilis* as a function of pH, with a total humic acid concentration of 0.1 g/l. Circular symbols represent experiments conducted with 0.5 g/l bacteria; triangles represent experiments conducted with 2 g/l bacteria. Model curves based on the adsorption model of Fein et al. (1999) (reaction 9 in text). Solubility curve (solid line) is a polynomial fit to the 'humic only' data set in Fig. 4.

presence of $10^{-3.5}$ molal Cd significantly reduces the solubility of the humic acid, removing approximately 90% of the humic from the solution.

3.2. Humic acid adsorption onto bacteria

The combined effect of solubility and the adsorption of humic acid onto the bacterial surface of *B. subtilis* is shown in Fig. 5 for a starting humic acid concentration of 0.1 g/l and two bacterial concentrations (0.5 and 2 g/l). Fig. 5 depicts the percentage of the total humic acid in the experiments that is removed from the solution after equilibration with the bacteria, and the 0.1 g/l humic solubility curve is shown in the figure as a solid curve. Experiments with both bacteria and humic acid present exhibit significantly higher humic acid removed relative to the solubility curve, indicating humic acid adsorption onto the bacterial surface. Humic acid adsorption is considerable from approximately pH 3 to 10. There is virtually no humic acid in solution under the low pH experimental conditions due to precipitation. Adsorption (viewed as the difference, or excess removed, between the data in Fig. 5 and the solubility curve) increases as the pH increases to 7. Above approximately pH 7, adsorption decreases with in-

Fig. 4. Humic acid solubility experiments in the presence of no Cd, 10^{-4} and $10^{-3.5}$ molal Cd. The graph depicts the percentage of the original concentration of humic acid (0.1 g/l) removed from solution due to precipitation and filtration under each pH condition.

creasing pH due to an increase in the repulsive force between the bacterial surface and the humic acid molecule. The amount of humic acid removed from solution increases as we increase the bacterial concentration from 0.5 to 2 g/l. The data from the humic acid–bacteria adsorption experiments show the same general trend of decreased adsorption with increasing pH as the model of Fein et al. (1999); however, there are noticeable differences between our data and the model curves from this study. These differences are likely due to varied experimental set-up and analytical procedures used in the two studies. Fein et al. (1999) used a different electrolyte ($NaNO_3$) in their experiments and their samples made basic to pH 12. Both of these differences may have attributed to the observed disparity; however, the overall observed adsorption behaviors are consistent.

3.3. Cd effects on humic acid adsorption

Humic acid adsorption by bacteria is relatively unaffected by the presence of Cd (Fig. 6) under most of the pH conditions studied. The observed adsorption from experiments containing either 10^{-4} or $10^{-3.5}$ molal Cd differ from the Cd-free experiments

Fig. 7. Cd concentrations for the humic–Cd adsorption experiments conducted at 0, 0.5 and 2.0 g/l bacteria. Model curves based on Cd adsorption model in the absence of humic acid, as described in text. (A) Total Cd concentration is $10^{-3.5}$ molal. (B) Total Cd concentration is 10^{-4} molal.

by less than 10%; a value within the range of our experimental uncertainty. At values greater than pH 8, the humic acid concentration in solution becomes dependent on the total Cd in solution, with less soluble humic acid at higher Cd concentrations. No significant ternary interactions occur under these high pH, high Cd concentration conditions. That is, the perceived increase in adsorption at high pH is independent of bacteria concentration and is most likely related to the formation of a Cd-hydroxide phase (to be discussed later).

3.4. Humic acid effect on Cd adsorption

Under metal-dominant conditions of $10^{-3.5}$ molal Cd, the amount of aqueous Cd in solution with 0.1 g/l humic acid is the same with or without bacteria in the system (Fig. 7A). The steep Cd concentration
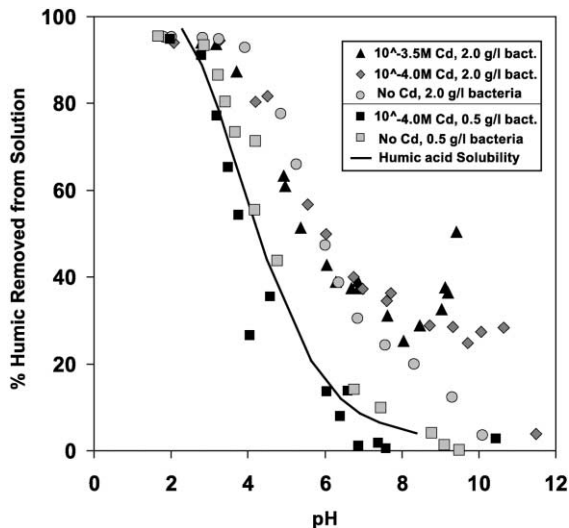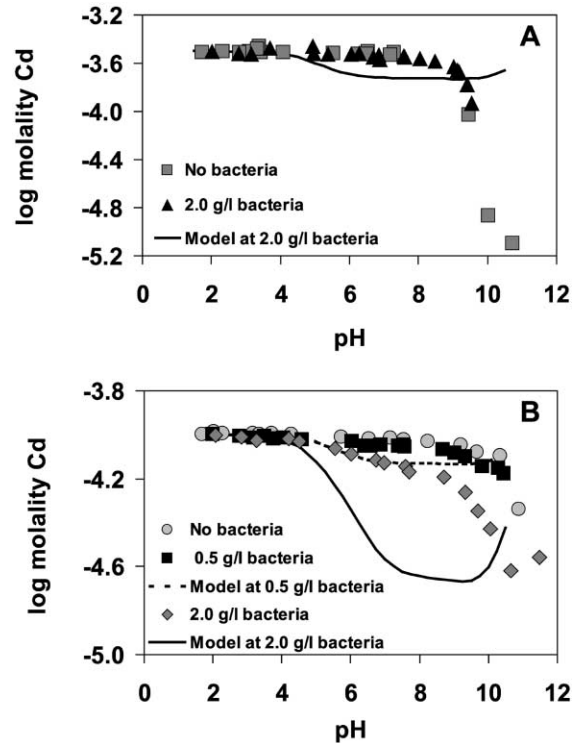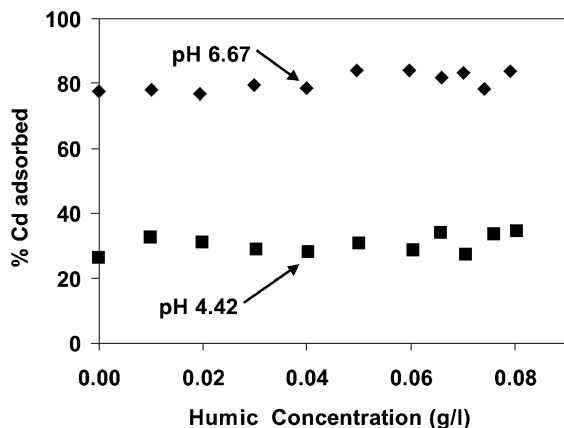

Fig. 6. Humic acid adsorption experiments in the presence of varying amounts of Cd at two bacteria concentrations. Solubility curve (solid line) is a polynomial fit to the 'humic only' data set in Fig. 4.

Fig. 8. Cd adsorption to *B. subtilis* at two pH values, as a function of the total humic concentration in the system. Experiments conducted at 10^{-4} molal Cd, 10 g/l bacteria at pH values of 6.67 and 4.42.

drop at approximately pH 9.2 is consistent with the formation of a Cd-hydroxide precipitate. With less Cd in the system (10^{-4} molal Cd, Fig. 7B), the data exhibit a trend of decreased aqueous Cd concentration with increasing bacteria concentration. This difference increases with increasing pH. In systems with higher concentrations of bacterial surface sites (Fig. 8; experiments with 10 g bacteria/l, $10^{-4.05}$ molal Cd and variable amounts of humic acid), we observed no significant trend in the amount of Cd adsorbed as a function of the total concentration of humic acid present in the experiments.

4. Discussion

4.1. Solubility experiments

We observed two different types of humic acid solubility behavior in the presence of Cd. Below pH 7, we observed no effect of Cd on humic solubility; above pH 7, high concentrations of Cd caused a significant decrease in the solubility of humic acid. The lack of a relationship between the amount of humic acid in solution below pH 7 with increasing Cd concentration implies that aqueous complexation, if it occurs, does not affect solubility, and that micelle formation, metal-induced coagulation, or me-

tal–humate precipitates do not affect the overall solubility under these conditions.

Above pH 7, in systems with high concentrations of both humic acid and Cd, the concentration of Cd and humic acid in solution significantly decreases with increasing pH, suggesting that humic acid and Cd are sequestered by a solid phase. Either they precipitate as a Cd–humic solid or humic acid adsorbs onto a Cd–hydroxide solid. Many Cd–organic precipitates have been documented (Seidell, 1940); however, the observed decrease in Cd in these experiments is much less than the decrease in humic concentration (on a molar basis). It is more likely that humic acid adsorbs strongly onto β-Cd(OH)$_{2(s)}$, which should precipitate in our experimental systems at pH values greater than 8.8 in 0.1 M electrolyte solutions containing $10^{-3.5}$ molal Cd (calculations based on data from Baes and Mesmer, 1976).

4.2. Cd–humic–bacteria experiments at constant total humic acid

Figs. 6 and 7 indicate that (1) humic acid adsorbs onto the bacterial surface to the same extent regardless of the amount of Cd in solution, and (2) humic acid significantly inhibits the adsorption of Cd to the bacteria. The models proposed by Fein et al. (1997) and Fowle and Fein (1999) document extensive Cd removal from solution via bacterial surface adsorption under similar solution conditions but without the presence of humic acid. The curves in Fig. 7 are constructed using a speciation model based on Eqs. (1)–(5),(10) and (11), metal hydration reactions from Baes and Mesmer (1976), and mass balance constraints using the metal and bacteria concentrations used in our experiments. These models predict 20–60% adsorption of Cd at pH values above 6. The model curves do not account for the formation of Cd–hydroxide solid phases and, consequently, they diverge from the data at pH values of greater than 9 or 10 where we expect Cd–hydroxide precipitation. In our humic-containing experiments with relatively high ratios of humic/bacteria sites (Fig. 7A,B), less Cd adsorbs onto the bacterial surface when humic acid is present in the system (relative to the model predictions).

There are two possible explanations for the diminished adsorption (relative to the model predictions)

observed in Fig. 7A,B: (1) that the humic acid blocks possible Cd adsorption sites or (2) that, under these humic/bacteria ratio conditions, a Cd–humate aqueous complex is present and sequesters Cd in solution. The first explanation is extremely unlikely. Given the concentration of humic adsorbed onto the bacterial surface, there should be abundant deprotonated carboxyl groups on the bacterial surface that are available for Cd adsorption. Also, the decrease in Cd adsorption continues to occur at pH values above 7, conditions at which little to no humic acid is adsorbed. Therefore, there must be an aqueous Cd–humate complex in solution that competes effectively with the bacterial surface for the available Cd. Competition between an aqueous organic anion and the negatively charged bacterial surface was also observed when measuring the effect of EDTA on the adsorption of Cd onto *B. subtilis* (Fein and Delea, 1999).

Under conditions with higher concentrations of bacterial sites (Fig. 8), the bacteria appear to effectively out compete the soluble humic acid for the available Cd. Under these relatively high concentrations of bacteria, virtually all of the humic acid adsorbs onto the bacterial surface, leaving little in solution to complex with the aqueous Cd. If Cd–humate complexation were significant in the experiments depicted in Fig. 8, the amount of Cd adsorbed would decrease with increasing humic acid concentration in the experiments. These experiments suggest that under these humic–bacteria–Cd concentrations, there is negligible interaction between adsorbing components, and that no ternary complexes form.

The existence of an aqueous Cd–humate complex is apparently inconsistent with the solubility data, which show no change in humic acid solubility in the presence of even high concentrations of aqueous Cd. The lack of change in solubility seems to suggest that there is no aqueous complexation between Cd and humate. However, we propose that humic acids are unlike mono-molecular organic molecules, which have solubilities that increase in response to aqueous complexation.

For a simple mono-molecular organic acid, any metal–organic aqueous complex formation would increase the overall solubility of the organic. For example, consider a generic, sparingly soluble, organic acid in the presence of a generic aqueous metal cation, Me^{2+}. If an aqueous metal–organic complex forms by the reaction:

$$Me^{2+} + Org^{2-} \rightleftharpoons MeOrg^0 \qquad (12)$$
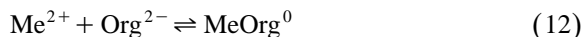
(where Org^{2-} represents the fully deprotonated aqueous organic acid anion), then the solubility of the organic acid would increase as a result of the lower Org^{2-} activity. At a constant pH, the activity of aqueous Org^{2-} is buffered by the solubility reaction,

$$H_2Org_{(s)} \rightleftharpoons Org^{2-} + 2H^+ \qquad (13)$$
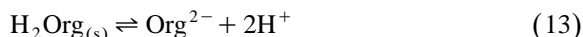
If the activity of Org^{2-} decreases through the formation of the $MeOrg^0$ complex, reaction (13) would be driven to the right and would thereby increase the aqueous solubility by an amount equal to the concentration of the $MeOrg^0$ complex. However, humic acid is not a mono-molecular organic acid but is best considered as a consortium of organic molecule species of varying molecular size, weight, and composition, with more species becoming soluble with increasing pH. Our data indicate that at least some of the species that are soluble form aqueous complexes with Cd, but that this complexation does not affect the solubility of the humic fraction, which remains insoluble.

These observations imply that the activities of the different fractions of humic acid are not interdependent. The thermodynamic activity of one aqueous humic fraction can decrease due to aqueous complexation with Cd^{2+}, but this does not affect the activity of any other aqueous humic fraction, and hence does not affect the solubilities of the other humic fractions either. Because of this, simple equilibrium reactions and traditional surface complexation modeling cannot be easily applied to ternary humic interactions. Humic acids represent a group of complex organic species that so far have not been sufficiently well characterized to allow for the application of an SCM in complex humic-bearing systems.

5. Conclusions

Aqueous Cd does not affect the adsorption of humic acid onto the bacterial surface of *B. subtilis*.

This result applies to both site-limited and abundant site conditions. Even in metal dominated systems, aqueous complexation between Cd and humic acid, if it occurs, does not limit humic acid adsorption in any perceptible way. Either the adsorption of Cd does not alter the electric double layer enough to allow for increased humic adsorption or the Cd–humate aqueous complex behaves in the same hydrophobic way as the uncomplexed humic acid and adsorbs non-specifically.

Aqueous Cd does not affect the solubility of humic acid, though humic acid does limit Cd adsorption onto bacteria surfaces under pH, bacteria, humic and Cd concentration conditions where significant concentrations of humic acid remain in solution. This apparently inconsistent behavior of the aqueous Cd–humate complex, of acting to decrease Cd adsorption onto the bacterial surface, while not affecting humic acid solubility, is not observed with simpler ionizable organic molecules. We propose that the behavior results from the heterogeneity of the humic acid. The solubility of one humic acid fraction is unaffected by the presence of the aqueous Cd–humate complex involving another humic acid fraction because the thermodynamic activities of the different fractions are not interdependent. At least some of the soluble humic acid, however, does form an aqueous complex with Cd in the mid pH range, thereby competing with the bacterial surface and decreasing Cd adsorption onto the bacterial surface.

The experimental results of this study demonstrate some of the complexities involved in quantifying the effects of humic acid on contaminant mobility in bacteria-bearing geologic systems. In some ways, humic acids behave in an analogous fashion to simple organic acids. They display reversible solubilities; their charging behavior can be modeled with discrete deprotonation constants; they adsorb to bacterial surfaces primarily through hydrophobic attraction; and in solution, they can effectively compete with bacterial surfaces to form complexes with available aqueous metal cations, dramatically altering the adsorption, speciation, and ultimate fate of those cations. However, due to their multi-component nature, humic acids are much more difficult to fit into traditional surface complexation models. Our results show that the thermodynamic activities of different humic fractions appear to be unrelated through mass

action laws, thereby causing behavior that is more complex than that observed for simpler single-component organic acids. Until these complexities are better understood and quantified, our understanding of the effects of humic acids on bacterial adsorption will remain qualitative.

Acknowledgements

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