

Significance of ternary bacteria–metal–natural organic matter complexes determined through experimentation and chemical equilibrium modeling

David Borrok *, Keith Aumend, Jeremy B. Fein

Department of Civil Engineering and Geological Sciences, University of Notre Dame, 156 Fitzpatrick Hall, Notre Dame, IN 46556, United States

Received 30 May 2006; received in revised form 19 October 2006; accepted 26 October 2006

Editor: L.M. Walter

Abstract

In this study, we conducted experiments that tested for the existence of ternary surface complexes involving bacteria, metal cations and natural organic matter (NOM). We performed a variety of batch complexation experiments in single (NOM only), binary (*Bacillus subtilis*–metal, NOM–metal, *B. subtilis*–NOM), and ternary (*B. subtilis*–metal–NOM) systems in order to determine the significance of ternary complexation. We conducted experiments as a function of 1) pH, 2) component concentrations, 3) fraction of NOM (humic, fulvic, or bulk), and 4) metal of interest (Pb, Cu, Cd, and Ni). Our investigative approach was to quantify the differences between the binary and ternary experimental systems under these different conditions. The concentration of NOM bound in ternary form was calculated directly from experimental data by comparing binary and ternary systems. The concentration of metal bound in ternary form was calculated using chemical equilibrium modeling.

Experimental results demonstrate that bacteria–metal–NOM complex formation is a rapid, fully-reversible chemical process. The pH of the aqueous system is the biggest factor in the stability, and therefore significance of ternary complexes. As pH decreases, bacteria–metal–NOM complexes become more stable. The fraction of NOM involved and the identity of the aqueous metal cation have a comparatively modest impact on the stability of ternary complexes. All NOM fractions form ternary complexes to similar extents at circumneutral pH, but humic acid becomes the dominant NOM fraction in ternary complexes at low pH. The abundance of humic acid in ternary form is greatest in experimental systems with Ni or Cd and less in systems with Pb and Cu.

These observations suggest that bacteria–metal–NOM complexes are abundant under the conditions present in most natural waters. Hence, ternary complexes may impact the mobility of aqueous metal cations in natural systems by changing dissolved NOM–metal complexes to colloidal bacteria–metal–NOM complexes.

© 2006 Elsevier B.V. All rights reserved.

Keywords: Bacteria; Ternary; NOM; Humic; Metals; Adsorption

1. Introduction

Both bacteria and natural organic matter (NOM) can affect the speciation of metal cations in near-surface environments through complexation reactions (e.g., Beveridge and Fyfe, 1985; Cabaniss and Shuman,

* Corresponding author. Present address: U.S. Geological Survey, MS 964D, Denver Federal Center, Denver, CO 80225.

E-mail address: dborrok@usgs.gov (D. Borrok).

1988; Beveridge, 1989; Xue and Sigg, 1999). Binary ‘bacteria–metal’ and ‘NOM–metal’ complexes can control the chemistry, fate, toxicity, and mobility of metals (Tornabene and Edwards, 1972; Ledin et al., 1996; Ma et al., 1999; Tortell et al., 1999; Fein, 2000; Richards et al., 2001). For example, bacteria attached to mineral surfaces can immobilize metals (Malard et al., 1994), while bacterial cells and dissolved NOM molecules in the water column can enhance metal mobilities through complexation (Harvey et al., 1989; Schmitt et al., 2003). However, the adsorption behavior of a metal in a system containing both bacteria and NOM may be significantly different from that predicted based solely on stability constants for the binary bacteria–metal and NOM–metal complexation reactions due to the co-adsorption of metals and NOM onto bacteria to form distinct ternary surface complexes. These ternary complexes can dramatically alter the mobilities and biogeochemical behavior of both the metal and organic ligands involved. For example, the intimate association of bacteria and NOM as a ternary complex may affect the consumption of NOM by bacteria as a carbon substrate and/or the utilization of NOM as an electron shuttling vehicle (Royer et al., 2002; Kappler et al., 2004). Despite the potential importance of ternary bacteria–metal–NOM complexes, there are few studies that have investigated mixed metal–NOM–bacteria systems.

Most previous complexation studies involving bacteria and/or NOM focus exclusively on binary systems, investigating either: 1) the complexation of bacteria or NOM with metals, 2) the sorption of bacteria or NOM onto mineral surfaces, or 3) the sorption of NOM onto bacterial surfaces. Bacterial cell walls contain organic acid functional groups (carboxyl, phosphoryl, hydroxyl, and amine) that deprotonate as a function of pH and can bind a range of aqueous metal cations (e.g., Beveridge and Murray, 1980; Fein et al., 1997; Small et al., 1999; Yee and Fein, 2001; Kulczykcki et al., 2002; Borrok et al., 2004a). Similarly, NOM (particularly humic and fulvic range macromolecules) are rich in carboxylic and phenolic functional group sites that deprotonate as a function of pH and also bind aqueous metal cations (e.g., Brady and Pagenkopf, 1978; Browne and Driscoll, 1993; Plette et al., 1995; Benedetti et al., 1996; Tipping et al., 2002). Due to functional group deprotonation reactions, both bacterial surfaces and NOM molecules possess a net negative charge even at very low pH values, and become increasingly more electronegative as pH increases (Van der Wal et al., 1997; Ritchie and Perdue, 2003; Yee et al., 2004; Fein et al., 2005; Borrok et al., 2005). Hence, the extent of complexation of metal

cations onto both bacteria and NOM increases with increasing pH due to the increased deprotonation of functional group sites with increasing pH. This pH-dependent complexation behavior has been quantified for a variety of bacteria–metal (e.g., Fein et al., 1997; Daughney et al., 1998; He and Hebo, 1998; Small et al., 1999; Espisito et al., 2001; Haas, 2001; Sokolov et al., 2001; Yee and Fein, 2001; Ngwenya et al., 2003; Borrok et al., 2004a) and NOM–metal (see Milne et al., 2003 for a review) systems. Because bacteria and NOM molecules bind metals as surface organic-acid complexes, the observed complexation behavior for specific metal cations can be directly linked to the known stabilities of similar aqueous metal–organic ligand complexes (e.g., Fein et al., 2001; Milne et al., 2003).

Compared with metal–bacteria adsorption and metal–NOM complexation studies, there have been significantly fewer studies that have investigated the sorption of NOM onto bacterial surfaces. Esparza-Soto and Westerhoff (2003) have shown that both humic and fulvic acid NOM fractions sorb strongly to activated sludge biomass, and that sorption increases as pH decreases, likely in response to hydrophobic forces. Carlson and Silverstein (1997) have demonstrated that significant quantities of lake water NOM sorb to biofilm-coated reactor media at a single pH of ~ 7.2 . Similarly, Johnson and Logan (1996) found that NOM sorbed to the surface of a rod-shaped, Gram-negative bacterium at a single pH of ~ 7.7 , resulting in enhanced bacterial transport through quartz media. In addition to these studies, both humic and fulvic NOM fractions have been shown to sorb to the surface of the Gram positive bacterium, *B. subtilis*, most extensively at low pH, with decreasing sorption with increasing pH (Fein et al., 1999; Wightman and Fein, 2001; Maurice et al., 2004). Maurice et al. (2004) further demonstrated that sorption of NOM onto *B. subtilis* caused significant molecular weight fractionation, as the more hydrophobic, higher molecular weight fractions preferentially adsorbed to the bacterial surface. The adsorption and fractionation behaviors of NOM suggest that the adsorption of NOM onto bacterial cell walls is primarily controlled by hydrophobic attraction under low pH conditions where functional groups on both bacteria and NOM are dominantly protonated and neutrally-charged. Electrostatic repulsion causes the decrease in interaction with increasing pH and extent of deprotonation.

Ternary, or three component complexes have been studied mainly in mineral–metal–organic ligand systems (e.g., see Schindler, 1990; Fein, 2002, for reviews). Very few studies have investigated ternary complexation involving bacterial surfaces. Wightman and Fein (2001)

found no evidence of ternary interactions in an experimental system with Aldrich humic acid, Cd^{+2} , and *B. subtilis*; however, experiments were conducted over a limited range of component concentrations, using a humic acid that is probably not representative of most in the environment. Conversely, Frost et al. (2003) found that significant amounts of fulvic acid– Cd^{+2} –*B. subtilis* complexes formed at pH values greater than 6.0, and that these ternary complexes became more abundant as the concentrations of Cd or fulvic acid were increased. Similarly, Esparza-Soto and Westerhoff (2003) demonstrated that the presence of Ca^{+2} enhanced the adsorption of NOM onto activated sludge biomass under the tested conditions at pH 6.0 and 8.0. These studies highlight a number of remaining research issues: (1) if and how the different molecular weight fractions of NOM influence the formation of ternary complexes, (2) how this behavior might vary as a function of the specific metal involved in each complex, (3) how the ratios of component concentrations impact the extent of ternary formation, and (4) how the extent of ternary complexation changes over a broad pH range.

The goal of this study is to estimate the importance of bacteria–metal–NOM complexes in natural systems and to elucidate the mechanisms of their formation. Toward this end, we conduct batch Pb, Cu, Cd, and Ni complexation experiments in binary (control) and ternary systems using the Gram positive bacterium, *B. subtilis*, and a soil humic acid, a stream fulvic acid, or bulk Suwannee River NOM. We test the impact of pH, type of NOM, type of metal, and bacteria:metal:NOM concentration ratio on the formation and stability of bacteria–metal–NOM complexes. We are able to detect the formation of ternary complexes by measuring both the extent of NOM and metal adsorption onto the bacterial surface in binary and ternary systems and comparing the experimental results. Chemical equilibrium modeling is used to quantify the thermodynamic stabilities of the important binary complexes in order to estimate the extents of ternary metal binding. Time-based experiments are also performed to determine the kinetics and reversibility of ternary formation.

2. Materials and methods

2.1. Bacteria and Growth Conditions

B. subtilis cells were initially cultured in 3 mL of trypticase soy broth (TSB) with 0.5% yeast extract for 24 h at 32 °C, then transferred to 1 L of broth of the same composition and grown for another 24 h at 32 °C. Bacterial cells were harvested from growth media by

centrifugation, transferred to test tubes, and washed five times in 0.1 M NaClO_4 , the same electrolyte used in each experiment. NaClO_4 was used because the perchlorate anion does not form complexes to an appreciable extent with H^+ , Cu^{+2} , Pb^{+2} , Ni^{+2} or Cd^{+2} under the experimental conditions. After each wash, bacteria were centrifuged for 5 min at 8000 rpm (~7150 g) to form a pellet at the base of the test tube and the electrolyte was discarded. Bacteria were re-suspended in fresh electrolyte solution for each subsequent wash. After the final wash, the bacteria were placed in weighed test tubes and centrifuged (7000 rpm [~5500 g] at 25 °C) for 1 h, stopping three times to decant all supernatant, and the mass of the moist bacterial pellet was determined. The mass recorded during this step is the wet mass we report throughout this study, and is approximately 8 times more than the dry mass of the bacteria (Borrok et al., 2005). The wet bacterial pellet was immediately used in experiments. Although the bacterial cells remain visually intact after this treatment (when observed using an environmental scanning electron microscope, Borrok et al., 2004b), they are not expected to be metabolizing during experiments because of the lack of electron donors and nutrients and because of the short (<3 h) experimentation times.

2.2. NOM

The fulvic acid used in this study was an XAD-8 isolate (Aiken, 1985) from Nelson's Creek, an organic rich stream located at the University of Notre Dame's Environmental Research Center in northern Wisconsin. Isolation and elemental analysis of Nelson's Creek NOM were conducted by Huffman Labs, Golden, Colorado. The XAD-8 isolation procedure selects only the humic substances fraction of the organic material (Maurice et al., 2002), and in this case, the humic substances were dominantly fulvic acids with lesser amounts of humic acid. Hence, we refer to this material as 'fulvic acid' throughout this manuscript. The relative weight percentages of the elements C, H, O, N, and S are approximately 47.1, 4.3, 45.5, 1.2, and 0.5, and the material ash content is ~1.4% by weight. The fulvic acid isolate was transformed into a freeze-dried powder, using a LABCONCO freeze dry system.

The humic acid used in this study was Elliott Soil standard humic acid (ESHA) obtained from the International Humic Substance Society (IHSS). ESHA contains 58.13% C and has an ash content of 0.88% (www.ihss.gatech.edu). ESHA contains both hydrophobic and hydrophilic organic acids. The sample location,

detailed isolation procedures, and entire elemental composition for ESHA are provided by the IHSS (www.ihss.gatech.edu).

The bulk NOM used in this study was Suwannee River NOM (SRNOM) obtained from the IHSS. SRNOM contains 52.47% C and has an ash content of 7.0% (www.ihss.gatech.edu). SRNOM contains the full spectrum of dissolved organic acids, including the hydrophobic and hydrophilic fractions. The reverse osmosis procedure used to isolate SRNOM is non-discriminatory in that all of the dissolved organic matter is collected (Maurice et al., 2002). The high ash content of this sample is a reflection of this bulk isolation procedure. The sample location, detailed isolation procedures, and entire elemental composition for SRNOM are provided by the IHSS (www.ihss.gatech.edu). Both the ESHA and SRNOM were provided as desalted, freeze-dried powders. Measured quantities of freeze-dried powder of fulvic acid, humic acid, or bulk NOM were suspending in 0.1 M NaClO₄ electrolyte solutions for experimentation, as described below. In this study, we base all NOM measurements on the total mass of freeze-dried powder used in each experiment, not the total mass of carbon in each isolate.

2.3. NOM solubility experiments

The solubility of each NOM fraction in aqueous solution is unique, and varies as a function of pH. Hence, it was necessary to establish a baseline solubility in 0.1 M NaClO₄ for each NOM fraction so that we could determine under which ternary experimental conditions enhanced NOM removal (adsorption) occurred. NOM solubility experiments were conducted using 0.1 M NaClO₄ at room temperature (~22 °C) with either fulvic acid, humic acid, or bulk NOM. Two types of NOM solubility systems were tested: 1) systems absent of both metal and bacteria, and 2) systems absent of bacteria, but containing either Pb, Cu, Ni, or Cd. In this study, the soluble fraction of NOM is defined as that which passes through a 0.45 μm nylon filter.

The aqueous metals (Pb, Cu, Ni, or Cd) used in these solubility experiments and in all other experiments reported herein were diluted from 1000 ppm atomic absorption standards, and were prepared in 0.1 M NaClO₄ solution prior to the addition of NOM. Stock solutions were prepared by suspending weighed NOM material in a known volume of 0.1 M NaClO₄, with or without metal, and stirring until the NOM was homogeneously distributed. Ten mL aliquots of the homogeneous suspensions were transferred into individual batch reaction vessels. The pH of each vessel was adjusted to

the desired value using approximately 0.01 mL to 0.03 mL of 0.01 M or 0.1 M NaOH or HNO₃. The chosen pH ranges of these experiments are discussed in detail below. After pH adjustments, each reaction vessel was allowed to equilibrate on a rotating rack for 2 h, after which time the equilibrium pH of the reaction was measured (the 2 h equilibrium time was chosen based on kinetic experiments described below). Each sample was then centrifuged and filtered through a 0.45 μm nylon syringe filter membrane to remove insoluble NOM. The entire supernatant volume from the filtration process was basified to pH ~ 11.0 using concentrated NaOH to ensure NOM remained dissolved prior to analysis. The dissolved NOM was then analyzed (within 12 h) using a dual cell ultraviolet visible light (UV–VIS) spectrometer at a wavelength of 450 nm. NOM standards for the UV–VIS analysis were prepared coincidentally with original experimental stock solutions using an identical 0.1 M NaClO₄ matrix. The standard solutions were prepared simultaneously with the experimental solutions because the absorbance of NOM-rich solutions can change as a function of time. Standards were made by preparing and then diluting a concentrated, basified solution of NOM (the same NOM fraction used in the experiment). All standards were basified to pH ~ 11.0 to match the samples, and were kept at room temperature prior to analysis. New standards were prepared for each experiment. Control experiments (not shown) indicated that the uncertainty involved in UV–VIS measurements that can be attributed to the preparation of the standards was approximately ±4%, while analytical uncertainty was ±1% (based on repeated measurements of external standards). Additional control experiments (also not shown) were performed to check for possible effects of metal precipitation on UV–VIS measurements caused by basification. We found that no visible precipitation occurred during basification of these samples, and that re-filtering of samples after basification did not impact absorbance measurements. NOM solubility experiments were conducted using 0.1 g/L of each NOM fraction and 4 different metals (5 ppm Cd, 5 ppm Pb, 5 ppm Ni, or 4 ppm Cu).

2.4. Binary experiments

We conducted two different types of binary system adsorption experiments: 1) ones to measure the extent to which each NOM fraction adsorbs onto *B. subtilis* in the absence of aqueous metals, and 2) ones to measure the extent to which each metal adsorbs onto the bacteria in the absence of NOM. These experiments were conducted to establish the baseline extent of NOM and metal

sorption onto *B. subtilis* so that we could determine whether NOM and metal removal (adsorption) was enhanced under any of the ternary experimental conditions.

Weighed material (bacterial pellets and/or NOM) were suspended in the 0.1 M NaClO₄ electrolyte stock solution (with or without metal present) and gently stirred until homogeneously distributed. The pH of each stock solution was adjusted to a circumneutral value prior to the addition of the bacteria pellet to prevent shock from acidic conditions. The aqueous speciation and precipitation thresholds of each experimental system were evaluated prior to experimentation using thermodynamic constants from Smith and Martell (1987). The experimental pH ranges were adjusted to avoid conditions under which precipitation and/or the formation of abundant aqueous metal hydroxide species might interfere with the interpretation and modeling of the experimental results. Systems with Cd⁺² or Ni⁺² were adjusted to a maximum pH of ~8.5, while systems bearing Cu⁺² or Pb⁺² were adjusted to a maximum pH of ~6.5. Values of pH below ~3.5 and above 9.0 were avoided in all cases to prevent irreversible damage to bacterial cells (Borrok et al., 2004b). Ten mL aliquots of the homogeneous suspensions were transferred into individual batch reaction vessels. The pH of each vessel was adjusted to the desired value using 0.01 mL to 0.03 mL of 0.01 M or 0.1 M NaOH or HNO₃. After pH adjustments, each reaction vessel was allowed to equilibrate on a rotating rack for 2 h, after which time the equilibrium pH of the reaction was measured. Each sample was then centrifuged and filtered through a 0.45 μm nylon syringe filter membrane to remove bacteria or insoluble NOM. Experiments were conducted under the following conditions: (A) 1 g/L *B. subtilis*+0.1 g/L NOM (for fulvic acid, humic acid, and bulk NOM); (B) 1 g/L *B. subtilis*+5 ppm metal (for Cd and Pb); (C) 5 g/L *B. subtilis*+4 ppm Cu; and (D) 10 g/L *B. subtilis*+5 ppm Ni. The metal, bacteria, and NOM concentrations were chosen to be higher than in natural systems so that they were easily quantifiable, and the concentration ratios of the respective components were chosen so that adsorption would occur over the desired pH range.

For the binary bacteria–NOM experiments, the entire supernatant volume from the filtration process was basified to pH ~11.0 and was analyzed using UV–VIS, as described above. For the binary bacteria–metal experiments, the entire supernatant volume from the filtration process was acidified (concentrated HNO₃) to pH ~1.0 for preservation of dissolved metal prior to analysis using an inductively coupled plasma -optical emission spectroscopy (ICP-OES) technique with

matrix-matched standards. Analytical uncertainty for ICP-OES measurements was determined to be less than approximately ±2% based on repeated measurements of external standards run between every 3 unknowns.

2.5. Ternary experiments

Ternary experiments were performed at room temperature (~22 °C) with *B. subtilis*, NOM, and metal. Measured masses of bacteria and NOM were added to metal-bearing, 0.1 M NaClO₄ stock solutions. The ternary stock solutions were gently stirred until homogeneous, and 10 mL aliquots of the homogeneous solution were transferred to individual batch reaction vessels. The pH of each vessel was adjusted and then allowed to equilibrate for 2 h on a rotating rack before the final, equilibrium pH was measured. The samples were centrifuged and filtered (0.45 μm), and the supernatant was divided in half. Half was acidified to pH ~1.0 for preservation of dissolved metal prior to analysis using an ICP-OES, and half was basified to pH ~11.0 and run using the UV–VIS to determine the concentration of NOM remaining in solution.

Two groups of ternary experiments were conducted: 1) Group 1 experiments in which we tested the impact of different NOM fractions on the extent of ternary complex formation, and 2) Group 2 experiments in which we tested the impact of different metals on the extent of ternary complex formation. Group 1 experiments were conducted under the following conditions: (A) 1 g/L *B. subtilis*+5 ppm Pb or Cd+0.1 g/L humic acid, fulvic acid, or bulk NOM; (B) 10 g/L *B. subtilis*+5 ppm Ni+0.1 g/L humic acid, fulvic acid, or bulk NOM; and (C) 5 g/L *B. subtilis*+4 ppm Cu+0.1 g/L humic acid, fulvic acid, or bulk NOM. These conditions were chosen to correspond to the conditions used in the solubility and binary experimental systems to facilitate direct comparison of the experimental results.

Group 2 experiments, testing the impact of metals on ternary complexation, were conducted such that the molar concentration ratio of the ‘total bacterial surface functional group site concentration’ relative to the ‘total dissolved metal concentration’ relative to the ‘total NOM functional group site concentration’ was held constant at 22:1:8. By using identical stoichiometric ratios of the components, we isolated the effect that different metals have on the formation of ternary complexes. Note that only the molar ratios of the components in the Group 2 experiments are identical, not the overall molar component concentrations, and concentrations were changed to accommodate the analytical detection limits for individual metals of interest. The molar ratio of 22:1:8 was

chosen because it is a reasonable approximation of the ratios of bacterial sites, metal, and NOM sites, respectively, in many natural aquatic systems. The concentrations of bacterial surface and NOM functional group sites used to calculate this ratio were determined previously through modeling of potentiometric titrations ($\sim 4.9 \times 10^{-3}$ NOM sites/g, Borrok and Fein, 2004; and $\sim 3.1 \times 10^{-4}$ *B. subtilis* sites/wet g, Fein et al., 2005). These published values can be used to convert from the mass concentrations (that we report here) to molar component concentrations. Group 2 experiments were conducted under the following conditions: (A) 1.9 g/L *B. subtilis*+5 ppm Pb+0.04 g/L humic acid; (B) 6.7 g/L *B. subtilis*+5 ppm Ni+0.14 g/L humic acid; (C) 5 g/L *B. subtilis*+4 ppm Cu+0.1 g/L humic acid; and (D) 3.5 g/L *B. subtilis*+5 ppm Cd+0.07 g/L humic acid.

2.6. Ternary kinetics and reversibility experiments

Kinetics experiments were conducted to determine the rate and stability of ternary complexation so that an adequate equilibration time could be established for all other experiments. The reversibility of ternary formation was tested to determine whether the formation of bacteria–metal–NOM complexes is an equilibrium process. A ~ 250 mL bacteria–metal–NOM stock solution was formulated as described above using 10 g/L *B. subtilis*, 5 ppm Ni, and 0.1 g/L bulk NOM in 0.1 M NaClO₄ electrolyte. The room temperature stock solution was gently stirred using a magnetic stir bar and stir plate. The pH of the stock solution was manually adjusted and held at ~ 4.0 using 0.01 mL to 0.03 mL of 1.0, 0.1, or 0.01 M HNO₃ or NaOH. Ten mL aliquots of the solution were removed as a function of time and promptly centrifuged and filtered. The supernatant was divided in half, preserved, and analyzed for aqueous Ni⁺² and NOM using an ICP-OES, and UV–VIS, respectively, as described above. After ~ 2 h, the stock solution was rapidly adjusted to pH ~ 7.0 , and additional samples were removed as a function of time, centrifuged, filtered, and analyzed for dissolved Ni⁺² and NOM. After an additional 2 h, the pH was adjusted back to ~ 4.0 and samples were again removed as a function of time and analyzed.

3. Results and discussion

3.1. Behavior of NOM in single and binary systems

The results of NOM solubility experiments (both with and without metals present) are presented in Fig. 1a–c for fulvic acid, humic acid, and bulk NOM, respectively.

All of the NOM fractions exhibit decreasing solubility with decreasing pH, presumably due to coagulation caused by hydrophobic attraction of the molecules under protonated low-pH conditions. All NOM fractions are most soluble above pH ~ 5.0 , while solubility rapidly decreases from pH ~ 5.0 to 3.0 (the lowest pH values examined). Surprisingly, there is little difference among the solubilities of the fulvic acid, humic acid, and bulk NOM under the tested conditions. For example, above pH 5.0, about 6%, 2%, and 4% of the fulvic acid, humic acid, and bulk NOM, respectively, are removed through the filtering process, while approximately 30% of each fraction is removed at pH 3.5. We attribute these similarities to the fact that all NOM fractions tested contain some high molecular weight, aromatic/hydrophobic molecules that probably control the observed solubilities.

The solubilities of the fulvic and humic acid samples do not appreciably change in the presence of Cu, Ni, or Cd (Fig. 1a and b). However, above pH ~ 5.5 , the presence of Pb decreases the solubility of humic acid by $\sim 10\%$. This result was confirmed in duplicate experiments, and suggests that under these conditions Pb acts as a coagulant for the soil humic acid. Macromolecular functional groups have a potent affinity for complexing Pb, and we speculate that these Pb–NOM bonds may change the configuration of the macromolecules, perhaps wrapping them into insoluble bundles. The solubility of fulvic acid in the presence of Pb was not tested due to lack of sample material. Above pH 5.0, the solubility of bulk NOM decreases 5 to 10% in the presence of Cu, Ni, Cd, or Pb, with the largest decrease in the presence of Pb (Fig. 1c). It is unclear why the solubility of bulk NOM is impacted (albeit slightly) by the presence of these metals, while fulvic and humic acid isolates are not (excepting the impact of Pb on the solubility of humic acid).

Binary experimental systems with NOM and bacteria (absent of metal) were used to determine the extent of adsorption of NOM onto *B. subtilis* without the aid of a bridging metal cation (or without the effect of ‘charge neutralization’, which is discussed below). The solubility of each NOM fraction does not change in the presence of *B. subtilis* (Fig. 1a–c). Hence, under the experimental conditions, humic acid, fulvic acid, and bulk NOM do not adsorb to the surface of *B. subtilis* in the absence of aqueous metal cations. This observation appears to contrast with other studies, which have shown that the adsorption of NOM onto bacterial cells is possible in the absence of metal cations (e.g., Esparza-Soto and Westerhoff, 2003; Maurice et al., 2004). However, the ratio of bacteria to NOM concentrations

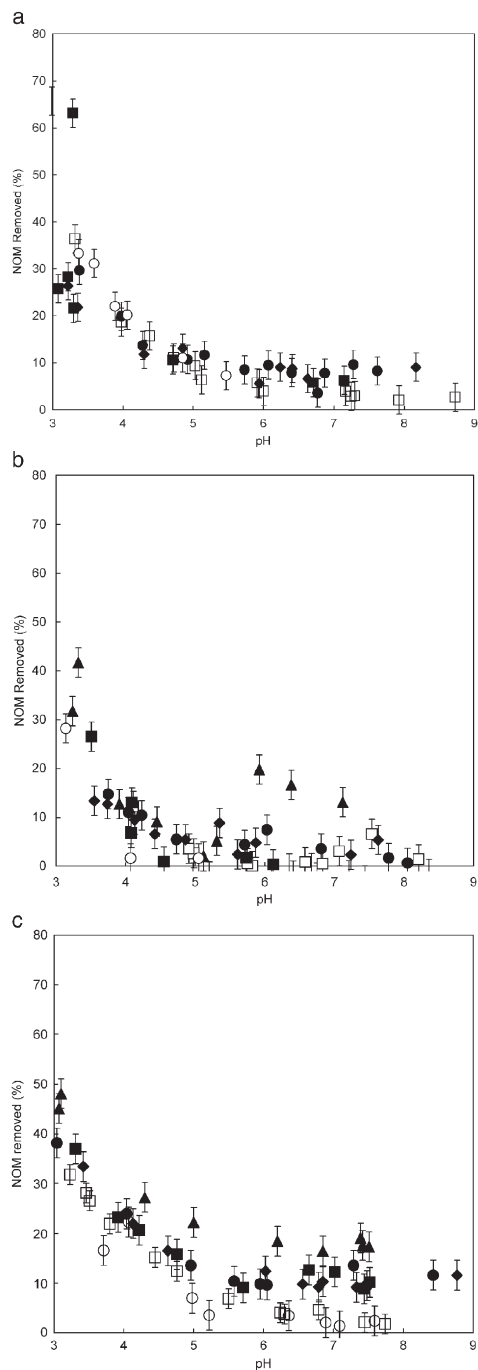


Fig. 1. Percentages of total NOM removed as a function of pH in solubility experiments. Symbols represent the following experimental conditions: 0.1 g/L NOM only (○), 0.1 g/L NOM+4 ppm Cu (■), 0.1 g/L NOM+5 ppm Pb (▲), 0.1 g/L NOM+5 ppm Ni (●), 0.1 g/L NOM+5 ppm Cd (◆), and 0.1 g/L NOM+1 g/L *B. subtilis* (□). All experiments were conducted in a 0.1 M NaClO₄ electrolyte solution. Identical experiments were conducted using (a) fulvic acid, (b) humic acid, and (c) bulk NOM. Error bars represent 1 sigma uncertainties based on the reproducibility of UV–VIS measurements.

used in these previous studies was higher by an order of magnitude or more compared to the concentration ratio used in our experiments. For example, Fein et al. (1999) and Wightman and Fein (2001) both demonstrate that under conditions similar to this study, very little Aldrich humic acid adsorbs to *B. subtilis* cells in the absence of metal cations. Significant adsorption of humic acid to *B. subtilis* (without metals) only occurred when the concentration of bacteria was increased.

3.2. Behavior of NOM in ternary systems

Group 1 ternary experiments test the impact of different NOM fractions on the formation of bacteria–metal–NOM complexes. The concentrations of fulvic acid, humic acid, and bulk NOM removed through centrifugation and filtration in the Group 1 experiments are presented in Fig. 2a–d for systems containing the metals Cd, Ni, Cu, and Pb, respectively. The percentage of NOM removed is attributable to either the adsorption of NOM onto the bacterial surface or to the presence of insoluble NOM residue (which was evaluated through solubility experiments). The solid curve in each figure represents the baseline solubility of bulk NOM with each of the respective metals. These curves are presented for visualization purposes only, and were constructed by fitting the bulk NOM solubility data from individual experiments (Fig. 1a–c) with a best-fitting polynomial. Using the baseline solubility data for each individual NOM fraction (in the presence of the respective metal), we can infer the extent of NOM ternary complexation without knowledge of the specific ternary stoichiometries. In each case, we qualitatively calculate the extent of ternary complexation by using the following relationship: [NOM bound as a ternary complex]=[NOM removed in ternary experiments]–[NOM removed in metal–NOM solubility experiments]. We can make this direct comparison because control experiments (described above) show that NOM does not adsorb to *B. subtilis* without metals present under the specific conditions of our study.

Both fulvic and humic acids form significant ternary complexes with bacteria in the presence of Cd (Fig. 2a). For example, there is a 10 to 20% difference between the baseline solubility data and the concentrations of humic and fulvic acid removed in the presence of Cd and *B. subtilis*. This difference suggests that 10 to 20% of the humic and fulvic acids are sorbed to *B. subtilis* as ternary complexes over the entire tested pH range. However, for bulk NOM, the baseline solubility and ternary removal measurements are nearly identical; suggesting that bulk NOM–Cd–bacteria complexes are

not significant under the tested conditions. Ternary complexation occurs mainly at low pH conditions for all NOM fractions in the presence of Ni and *B. subtilis*, and humic acid–Ni–*B. subtilis* complexes appear to be more stable than Ni–NOM–bacterial ternary complexes involving the other forms of NOM (Fig. 2b). For instance, ~15% of the humic acid is bound in ternary form at $\text{pH} \geq 6.5$, and this amount increases with decreasing pH to ~60% at pH 5.0. Fulvic acid and bulk NOM do not form ternary complexes to an

appreciable extent with Ni and *B. subtilis* at $\text{pH} \geq 6.5$, and only become significant at lower pH values, accounting for ~25% of the available NOM at pH 5.0 (Fig. 2b). All NOM fractions also form ternary complexes in the Cu system with increasing ternary complexation with decreasing pH. For example, at pH 6.5, only ~10% of the humic acid and bulk NOM are in ternary form, while ternary complexes with fulvic acid are negligible (Fig. 2c). Ternary complexes increase dramatically for fulvic, humic, and bulk NOM in the Cu

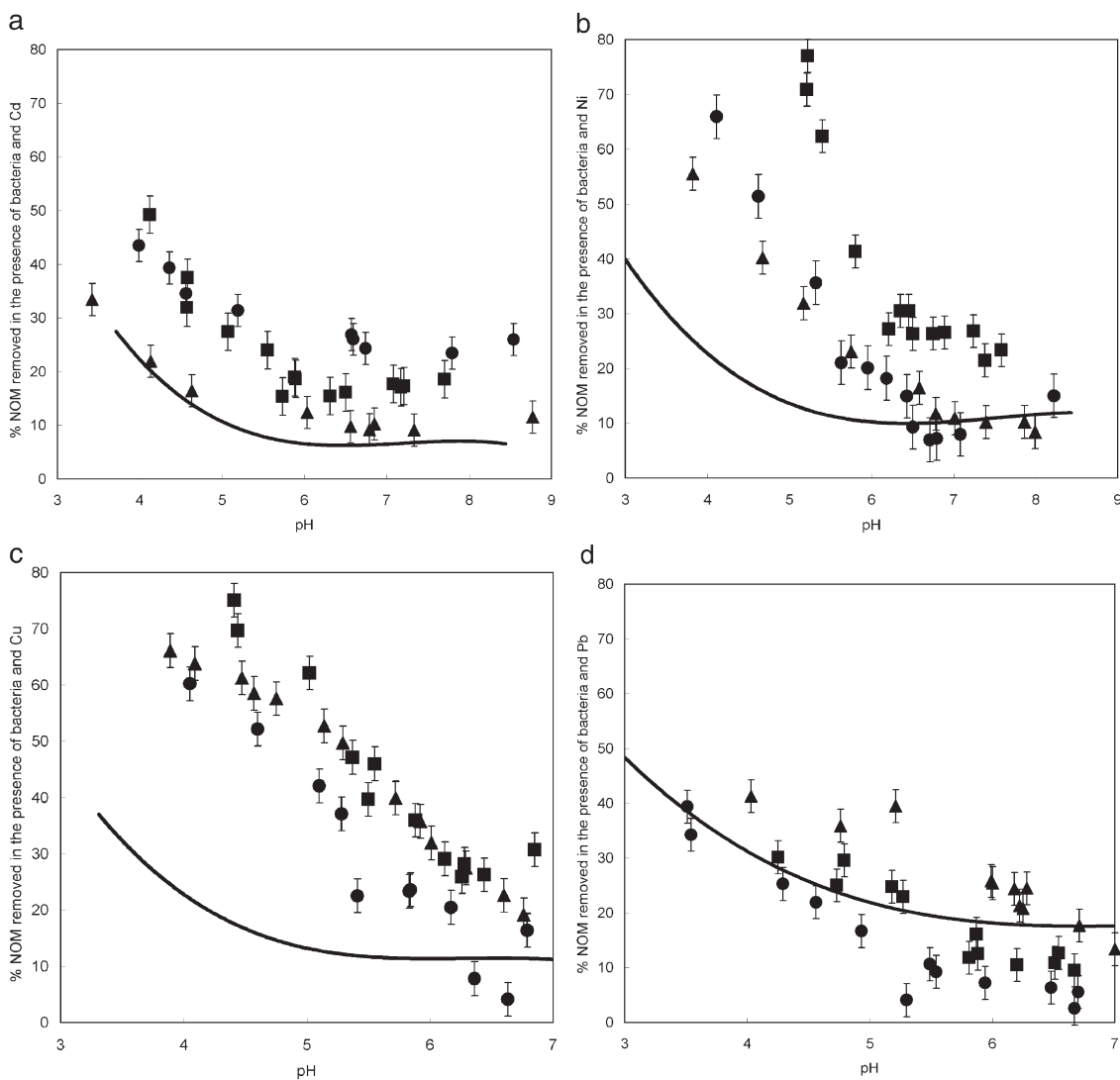


Fig. 2. Percentages of total NOM removed as a function of pH in Group 1 ternary systems. Symbols represent experiments conducted with humic acid (■), fulvic acid (●), and bulk NOM (▲). Experiments were conducted using (a) 5 ppm Cd + 1 g/L *B. subtilis*, (b) 5 ppm Ni + 10 g/L *B. subtilis*, (c) 4 ppm Cu + 5 g/L *B. subtilis*, and (d) 5 ppm Pb + 1 g/L *B. subtilis* in addition to 0.1 g/L of each NOM fraction in 0.1 M NaClO₄. For visualization purposes, the baseline solubility of bulk NOM in the presence of the respective metals is presented as a best-fit solid curve (solubility data for humic and fulvic acids are presented in Fig. 1). Error bars represent 1 sigma uncertainties based on the reproducibility of UV–VIS measurements.

system as pH values decrease. As observed in the Cd and Ni systems, humic acid forms ternary complexes with Cu and bacteria to slightly greater extents than do bulk NOM or fulvic acid over the entire pH range tested. (Fig. 2c). Only bulk NOM and humic acid form measurable ternary complexes in the Pb system (Fig. 2d). About 10% to 15% of the humic acid and bulk NOM form ternary complexes with Pb and *B. subtilis* over the pH range of ~4 to 6 (Fig. 2d). However, at higher pH (≥ 6.0) none of the NOM fractions form ternary complexes with Pb and *B. subtilis*, and in some cases the baseline solubility values are greater than the measured removal of NOM in the ternary systems (Fig. 2d). This result suggests that the addition of bacteria to the NOM–Pb system actually increases, albeit slightly, the solubilities of some NOM fractions. We speculate that the competition between binary Pb–bacteria complexes and Pb–NOM complexes in the ternary system acts to keep the concentrations of binary Pb–NOM complexes down, decreasing the coagulation effect observed in the same systems absent of bacteria.

The results of Group 1 experiments suggest that humic acid, fulvic acid, and bulk NOM are all capable of forming ternary complexes with a variety of metals. Ternary complexes for all NOM fractions are most stable under acidic conditions and become less abundant as pH increases. This observation is based mainly on Group 1 experiments with Cu and Ni, and is not as clear for experiments with Cd and Pb where less ternary complexation occurred. However, this observation is reaffirmed for Cd and Pb in the discussion of Group 2 experiments that follows below. The increase in abundance of ternary complexes with decreasing pH suggests that hydrophobic forces play a critical role in the formation of these complexes. Hydrophobic forces become dominant at low pH when the functional groups on the bacterial surface and the NOM are mostly protonated. The bacterial cell wall and NOM macromolecules become less electronegative, and interact less favorably with polar water molecules at lower pH. The higher molecular weight, more hydrophobic humic acid fraction generally forms ternary complexes to greater extents than do the fulvic acid or bulk NOM fractions at low pH, supporting this interpretation.

Bulk NOM contains higher concentrations of contaminant cations, has a higher ash content, and contains some less chemically reactive molecules (miscellaneous DOC) than the isolated humic and fulvic fractions (Maurice et al., 2002). Therefore, we expected fewer ternary complexes to form in systems with bulk NOM (when compared per total mass of material). However, this

was only the case in the Cd system. It is not clear why this is the case; however, it may suggest that Ni, Pb, and Cu form more stable ternary complexes than Cd with bulk NOM.

Ternary complexes may form in two distinct ways: 1) metals may act as a bridge, sharing a bond with both bacteria and NOM (i.e., electrostatic bonding processes), or 2) metals may help to neutralize the electronegative charges of bacteria and NOM through individual complexation reactions, enhancing hydrophobic interactions. This second option is analogous to neutralizing charge by lowering the pH of the system. It is not possible in our study to distinguish between these two ternary formation processes; however, it is likely that both mechanisms (electrostatic and hydrophobic) are at work to some extent.

Group 2 experiments isolate the impact of different metals on the extent of ternary complexation for systems with humic acid only. The results, presented in Fig. 3, demonstrate that under identical (and environmentally relevant) component concentration ratios, ternary complexes with bacteria can be the dominant chemical species for humic acid under low pH conditions. For example, more than 75% of the NOM was removed from solution in the Cu, Pb, Cd, and Ni systems at pH 4.5 (Fig. 3). For visualization purposes, the baseline solubility for humic acid in the presence of Cd is presented as a solid curve (Fig. 3). It is also apparent that humic acid forms ternary

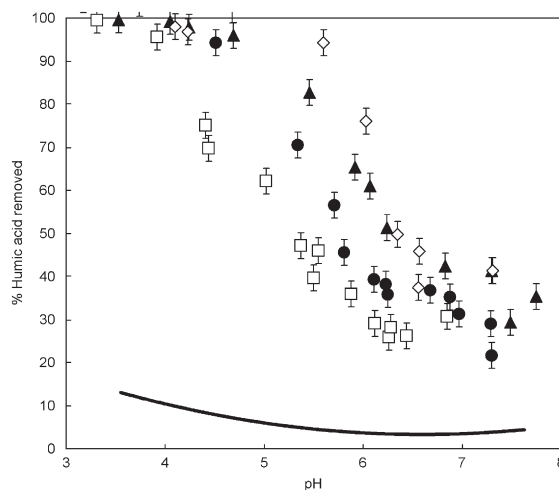


Fig. 3. Percentages of the total humic acid removed as a function of pH for Group 2 ternary systems. Experiments contained Cu (\square), Pb (\bullet), Ni (\blacktriangle), or Cd (\diamond) in addition to *B. subtilis* and humic acid in a 0.1 M NaClO₄ electrolyte. A molar stoichiometry of 22:1:8 for 'bacterial surface sites' to 'metal' to 'humic acid sites' was used in each experiment, with exact concentrations presented in the text. For visualization purposes, the best-fit solid curve for the solubility of humic acid in the presence of Cd is presented. Error bars represent 1 sigma uncertainties based on the reproducibility of UV–VIS measurements.

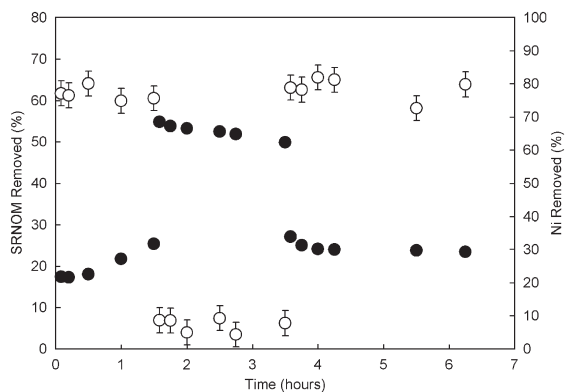


Fig. 4. Percentage of bulk NOM and Ni removed during kinetics/reversibility experiment. The experiment was conducted in a ternary system of 10g/L *B. subtilis*, 0.1 g/L bulk NOM, and 5 ppm Ni in a 0.1 M NaClO₄ background electrolyte. Concentrations of bulk NOM (O) and Ni (●) removed from solution were monitored as function of time at 3 different pH steps. Error bars represent 1 sigma uncertainties based on the reproducibility of UV–VIS measurements.

complexes to the greatest extents in systems with Cd and Ni, and to slightly lesser extents in systems with Pb and Cu. For example, at pH 6.5, ~30% of the humic acid was removed in the Cu system and ~40% was removed in the Pb system, while ~50% was removed in the Cd and Ni systems. Note that this comparison cannot be made for the Group 1 experiments, as they were conducted at significantly different stoichiometric concentration ratios of bacterial sites to metal to NOM sites. This sequence (Cu < Pb < Ni < Cd) is the reverse of the sequence expected if the overall affinity of each metal for bacterial surface or NOM functional groups is considered. For example, the stability constants for carboxylic bonds (using oxalate or citrate as an analog ligand) for these metals goes in the order Cd ≤ Ni < Pb ≤ Cu (Smith and Martell, 1987; also see Fein et al., 2001). This suggests that metals with lower affinities for binary complexation with NOM or bacteria will more readily facilitate the sorption of humic acid to bacteria than metals with higher binary affinities. We speculate that the strong binary NOM–metal and bacteria–metal complexes that form with metals like Pb and Cu tend to be more stable than ternary complexes with these same metals. Hence, abundant metals with lower affinities for binary complexation like Ni and Cd (or perhaps Ca and Mg in natural systems) may facilitate the sorption of NOM onto bacterial surfaces (i.e., form ternary complexes).

3.3. Kinetics and reversibility of ternary complexation

Fig. 4 presents the result of kinetics and reversibility experiments in the ternary, *B. subtilis*–Ni–bulk NOM

system. Open circles represent the concentration of NOM removed from the system as a ternary complex (or as insoluble residue), while closed circles represent the concentration of Ni removed from the system (bound to the bacterial surface in binary or ternary form). We can be certain that ternary complexes are forming in this experiment because significantly more NOM is adsorbed to bacteria under these ternary conditions than in equivalent binary experiments where Ni is absent (see above). The concentration of NOM equilibrates rapidly after each adjustment of pH, stabilizing in less than 5 min (Fig. 4). However, the concentration of Ni bound to the bacterial surface does not stabilize as rapidly, suggesting that the kinetics of Ni complexation in ternary form or Ni complexation in binary form (to the bacterial surface or NOM) is significantly slower. For example, the concentration of Ni removed from the ternary system increases from about 18% to 26% over 1.5 h at pH 4.0, and decreases from about 55% to 50% over 2 h at pH 7.0 (Fig. 4). The concentration of Ni bound to the bacterial surface stabilized more rapidly after the pH of the system was reversed back to the original value of 4.0 (Fig. 4). It is not possible to determine what the rate limiting reaction for Ni complexation is through this experiment. However, previous work has demonstrated that metal complexation to the bacterial surface and metal complexation to NOM in binary systems is rapid (Jin et al., 1996; Fowle and Fein, 2000), suggesting that ternary Ni complexation may be the rate limiting step. Despite slight differences due to kinetic effects, the concentrations of NOM and Ni complexed at pH 4.0 remain the same before and after the intermediate step at pH 7.0 (Fig. 4), demonstrating that ternary complexation is a reversible chemical reaction.

3.4. Metal complexation in binary and ternary systems

The concentration of NOM bound in ternary form was estimated directly from experimental data. However, it was not possible for us to directly estimate the concentrations of metals bound as ternary complexes from experimental data as we did for NOM. Although we can accurately measure the total concentrations of metals bound to the bacterial surface in each experiment, we have no method for distinguishing between metals bound as binary, ‘metal–bacteria’ complexes and ternary, ‘bacteria–metal–NOM’ complexes. Hence, in order to distinguish between these possibilities, we use chemical equilibrium modeling to predict the quantities of binary, ‘bacteria–metal’ and binary, ‘NOM–metal’ complexes in each experiment. In this way we can

predict the baseline extent of metal adsorption onto *B. subtilis* assuming that ternary complexes do not form, and compare this baseline to the raw data from the ternary experiments. The differences between the baseline (assuming binary interactions only) and the ternary data reflect the extent of metal binding attributable to ternary complexation. The concentrations of metals adsorbed to the bacterial surface in Group 1 experiments are presented for the binary (bacteria–metal) and ternary (bacteria–metal–NOM) experiments in Fig. 5a–d for Cd, Ni, Cu, and Pb, respectively. The

concentrations of metals adsorbed to the bacterial surface in Group 2 experiments are presented for the ternary (bacteria–metal–NOM) experiments in Fig. 6. The extents of binary (bacteria–metal) adsorption were not examined for Group 2 experimental conditions, but were instead estimated through modeling (see below).

3.4.1. Chemical equilibrium modeling framework

The chemical equilibrium model explicitly accounts for the complexation of protons and metals with the bacterial cell wall and NOM in our experimental

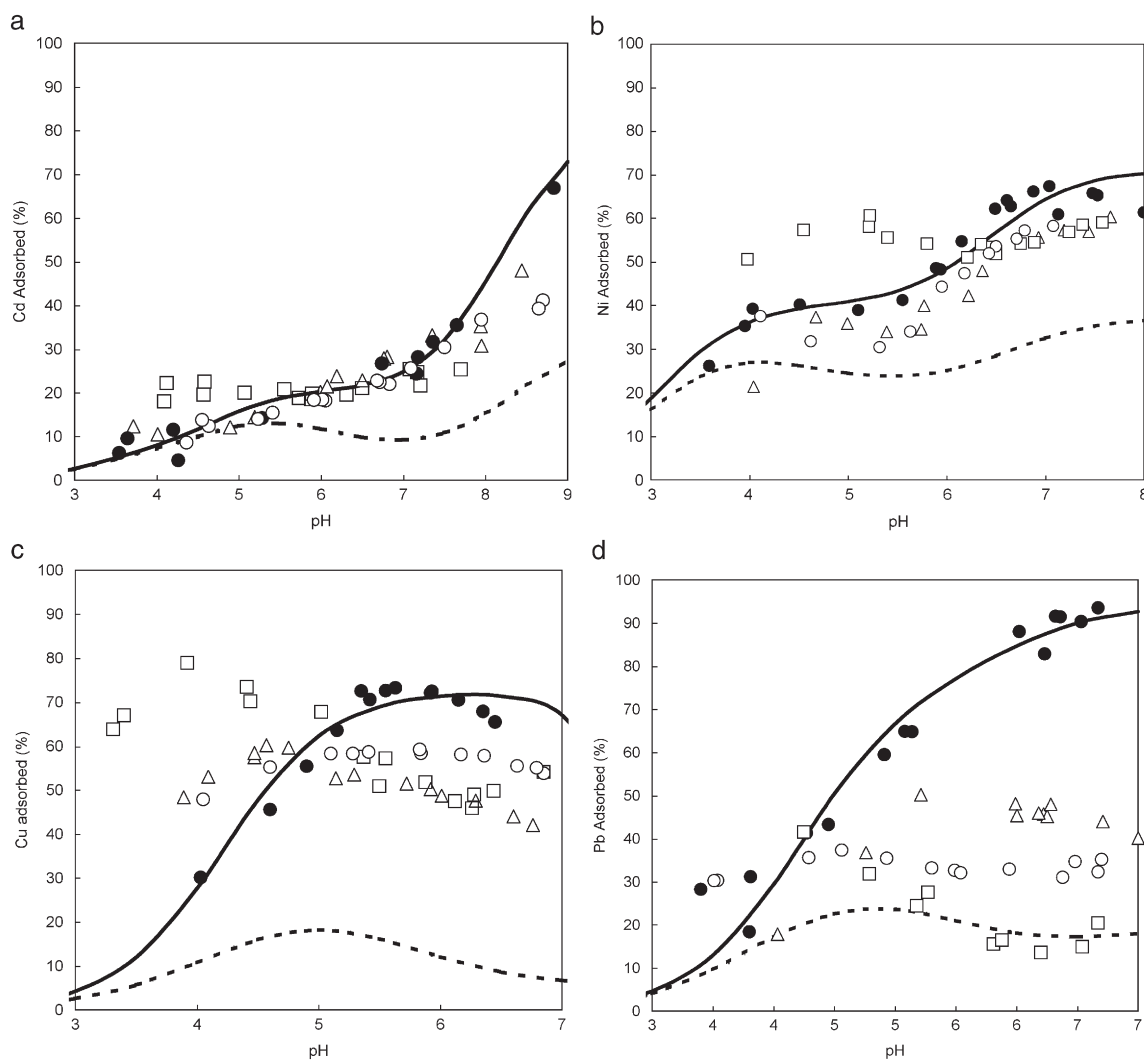


Fig. 5. Percentages of metal adsorbed to bacteria in Group 1 ternary systems. Experiments were conducted using (a) 5 ppm Cd+1 g/L *B. subtilis*, (b) 5 ppm Ni+10 g/L *B. subtilis*, (c) 4 ppm Cu+5 g/L *B. subtilis*, and (d) 5 ppm Pb+1 g/L *B. subtilis* in the presence of 0.1 g/L humic acid (□), 0.1 g/L fulvic acid (○), and 0.1 g/L bulk NOM (△). Binary *B. subtilis*–metal data (●) are also presented in each figure. The concentrations of metal and bacteria in the binary systems are the same as in the respective ternary systems. All experiments were conducted in a 0.1 M NaClO₄ electrolyte. Solid curves represent the model fits for metal complexation in the binary, *B. subtilis*+metal experiments, while the dashed curves represent the predicted model fits for ternary experiments neglecting ternary complexation. The difference between the dashed curves and the experimental ternary data represents the approximate concentrations of metals bound in ternary form.

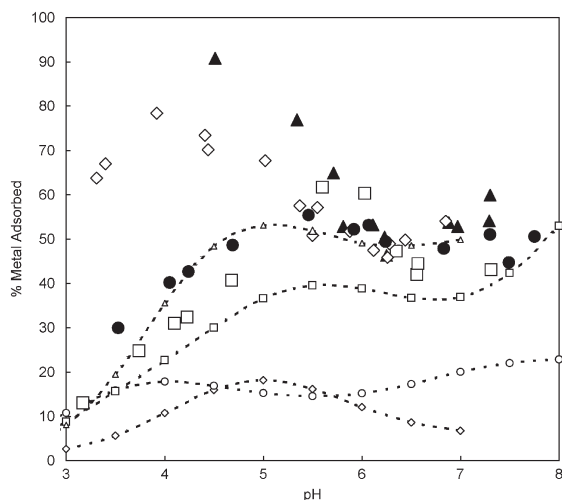


Fig. 6. Percentages of metal adsorbed to bacteria in Group 2 ternary systems. Experiments were conducted using Cu (\diamond), Pb (\blacktriangle), Ni (\bullet), and Cd (\square) under identical ternary stoichiometries of 22:1:8 for ‘bacterial surface sites’ to ‘metal’ to ‘humic acid surface sites’. Specific component concentrations are presented in the text. Dashed curves are predicted model fits for ternary experiments neglecting ternary complexation. Open symbols with the dashed curves correspond to the shapes of the larger solid symbols used for each metal.

systems. The organic acid functional groups (present either on bacterial surfaces or NOM) can be approximated as a number of discrete monoprotic acids (after Borrok et al., 2005). Each monoprotic acid undergoes the following deprotonation reaction:



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

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

where $[\text{R} - \text{A}_i^-]$ and $[\text{R} - \text{A}_i\text{H}^\circ]$ represent the concentrations of deprotonated and protonated sites, respectively, and a_{H^+} represents the activity of protons in the bulk solution. Metal complexation with the deprotonated forms of the monoprotic acids can be expressed as:



where M^{+2} is the aqueous divalent metal cation of interest (Pb, Cu, Ni, or Cd). The stability constant, K , for this reaction is given by:

$$K = \frac{[\text{R} - \text{A}_i(\text{M})^{+1}]}{a_{\text{M}^{+2}}[\text{R} - \text{A}_i^-]} \quad (4)$$

where $[\text{R} - \text{A}_i(\text{M})^{+1}]$ is the concentration of the metal–ligand complex of interest, and $a_{\text{M}^{+2}}$ is the aqueous activity of the metal cation. This modeling framework and approach are simplistic, but have been shown to be effective in approximating the metal and proton binding behavior of both NOM and bacteria over a broad range of conditions (e.g., Westall et al., 1995; Borrok and Fein, 2004; Borrok et al., 2005; Fein, 2006). Electric double layer effects are not explicitly accounted for in this model because we have found that they are generally negligible in comparison to other uncertainties (Borrok and Fein, 2005) and we have no way of constraining them as we performed all experiments at a single ionic strength.

3.4.2. Development of modeling parameters

Experimental data from the binary metal–bacteria systems were used to calculate the stability constants for the important metal–bacterial surface complexes. The buffering capacity of *B. subtilis* can be described using 4 discrete ligand sites with concentrations of 8.1×10^{-5} , 1.1×10^{-4} , 4.4×10^{-5} , and 7.4×10^{-5} mol/g, and acidity constants (pK_a) of 3.3, 4.8, 6.8, and 9.1, respectively (Fein et al., 2005). We use the FITEQL 4.0 modeling program (Herbelin and Westall, 1999), along with the average acidity constant and site concentration values, to determine the Pb–, Cu–, Cd–, and Ni–bacteria surface stability constants from the binary, metal–*B. subtilis* adsorption data. In each case, we determine the minimum number of surface ligands that are required to bind metal such that the observed adsorption behavior is accounted for, and solve for the values of the corresponding stability constants for each adsorption reaction (e.g., Eq. (4) using FITEQL. Metal speciation is accounted for in each model using the equilibrium constant values from Smith and Martell (1987) for aqueous metal–hydroxide species. We test models for adsorption onto all 4 surface ligands individually and in combination to determine the best fit to the experimental data. The best fitting model can be established using the error estimates within FITEQL 4.0, which are based on minimizing the weighted sum of squares of the difference functions for each fit (see Herbelin and Westall, 1999).

The best fit model for Cd adsorption onto *B. subtilis* requires complexation with the surface ligands with pK_a values of 3.3, 4.8, and 9.1. Cadmium binding with the ‘6.8 pK_a ligand’ could not be constrained because its inclusion did not provide an improved fit to the experimental data. Note that stability constants for metal complexation with all ligands can be constrained given additional experimental data collected under a variety of bacteria:Cd concentration ratios. However, the goal of

this study is not to derive a set of ‘exhaustively-tested’ stability constants for metal binding onto each ligand of *B. subtilis*, but to derive stability constants that accurately describe the exact experimental conditions in this study for comparison to ternary experiments. Hence, this model accomplishes our goal by accurately fitting the Cd adsorption data in Fig. 5a (solid curve). The logarithm of the Cd binding stability constants are 3.3, 3.6, and 5.4, for ligands with pK_a values of 3.3, 4.8, and 9.1, respectively. The best fit model for Ni adsorption onto *B. subtilis* requires complexation with the surface ligands with pK_a values of 3.3 and 6.8 only, with log stability constants of 3.3 and 4.0, respectively (fit shown in Fig. 5b). The best fit model for Cu adsorption onto *B. subtilis* requires complexation with the surface ligand with a pK_a value of 4.8 only, with a log Cu stability constant of 4.0 (Fig. 5c). The best fit model for Pb adsorption onto *B. subtilis* requires complexation with the surface ligands with pK_a values of 4.8 and 6.8 only, with log stability constants of 4.7 and 6.2, respectively (Fig. 5d).

In order to develop stability constants for the important metal–NOM complexes, we use experimental data collected by other researchers and compiled by Milne et al. (2003). Borrok and Fein (2004) demonstrated that despite the inherent heterogeneity among different NOM molecules, the proton and metal binding constants are relatively insensitive to the specific molecule involved. Based on this observation, we model all of the NOM molecules in this study with a single set of averaged acidity constants, site concentrations, and metal stability constants. The buffering capacity of an average NOM molecule can be described using 4 discrete ligand sites with concentrations of 1.5×10^{-3} , 1.6×10^{-3} , 8.2×10^{-4} , and 9.8×10^{-4} mol/g, and acidity constants (pK_a) of 3.1, 4.7, 6.6, and 9.0, respectively (Borrok and Fein, 2004). Moreover, average log stability constants for Cd–NOM complexes are 2.9, 3.6, 4.9, and 5.5 for these four sites, respectively (Borrok and Fein, 2004). Here, we used the existing average NOM pK_a values and site concentrations in conjunction with existing Ni–, Pb–, and Cu–NOM complexation data from Milne et al. (2003) to constrain our calculations within FITEQL.

We selected suitable NOM–metal binding datasets from Milne et al. (2003), with selection criteria identical to those described previously for Cd-complexation data sets (Borrok and Fein, 2004). Eight NOM–Cu datasets, 7 NOM–Pb data sets, and 1 NOM–Ni dataset were selected, and an additional NOM–Ni dataset from Zhou et al. (2005) was also utilized. We retained the numbering convention of Milne et al. (2003) for ease of reference, and a brief description of each dataset is included in Table 1. Metal hydrolysis products, as well as metal

precipitates, were considered in the speciation calculations, with equilibrium constants taken from Smith and Martell (1987). Because of the limited pH range of each individual dataset, stability constants could only be constrained for metal complexation with a limited number of the discrete ligand sites at a time. Best fit models were developed for each individual data set (model fits and data not shown; see Borrok and Fein, 2004 for an example). Stability constants for each of the best-fitting metal-binding models, their means, and standard deviations are presented in Table 1. Ideally, more NOM–metal binding data would be available to better constrain stability constants over a broad pH range. However, even with the paucity of data, the stability constants presented in Table 1 are remarkably similar given the variety of soil and aquatic NOM fractions used to derive them. For example, the calculated 1σ uncertainties for the stability constants for Cu, Pb, and Ni complexation (with the $pK_a=4.7$ site) are 0.2, 0.5, and 0.5 log units, respectively (Table 1). This suggests that, to a first approximation, these averaged constants are likely to yield reasonable approximations of the metal complexation behavior of the NOM fractions used in our experiments.

A joint binary model that describes the competition between bacteria and NOM for aqueous metal cations was developed utilizing the stability constants presented here. This ‘ternary-free’ model predicts the extent of adsorption expected in each ternary system neglecting ternary surface complexation but accounting for competition between the binary, bacteria–metal and NOM–metal species. In this approach, observed extents of adsorption onto *B. subtilis* that are in excess of the predicted extents of adsorption represent strong evidence for the formation of ternary surface complexes. This approach is necessary in order to qualitatively demonstrate the existence of ternary complexes and the pH ranges over which they are stable. This approach assumes that 1) the stability constants developed here (and those developed previously by Borrok and Fein (2004), and Fein et al. (2005)) provide a reasonable approximation of the speciation of the system if ternary complexes are not present, and 2) that the extent of NOM–metal complexation is similar for each fraction of NOM tested. In other words, we make the simplifying assumption that the average NOM stability constants that we calculated from the data of Milne et al. (2003) apply equally to the humic acid, fulvic acid, and bulk NOM used in these experiments. We believe this is a reasonable approximation because the NOM–metal binding stability constants were developed using a diverse array of datasets collected from experiments with humic and fulvic acids isolated from a variety of

Table 1
Pb, Ni, and Cu complexation constants for NOM (monodentate complexes onto deprotonated sites — see Eq. (4))

Dataset	Location	Metal, concentration range (ppm)	Ionic strength	Site 1 (pK _a 3.1)	Site 2 (pK _a 4.7)	Site 3 (pK _a 6.6)	Site 4 (pK _a 9.0)
FCu1	Suwannee R.	Cu, 0–4	0.01	NA	5.5	NA	NA
FCu3	Podzol soil	Cu, 13–14	0.1	NA	5.0	NA	NA
FCu5	Podzol soil	Cu, 1–17	0.1	NA	5.2	NA	NA
FCu8	IHSS FA4	Cu, 1–3	0.1	NA	4.7	6.2	NA
HCu5a	Summit Hill	Cu, 0.7	0.1	NA	5.0	6.7	NA
HCu5b	Summit Hill	Cu, 3	0.1	NA	4.7	6.0	NA
HCu5c	Summit Hill	Cu, 3	0.1	NA	NA	6.7	NA
HCu8	Eliot Silt	Cu, 0–55	0.01	4.3	4.8	NA	NA
Average		Cu		4.3	4.9	6.4	NA
S.D.				NA	0.2	0.4	NA
FPb1	Oyster R.	Pb, 0–41	0.1	NA	4.8	6.6	NA
FPb2	Armadale	Pb, 2–10	0.1	4.0	5.1	NA	NA
FPb3	Podzol soil	Pb, 1–15	0.1	NA	5.1	6.8	NA
FPb4	Podzol soil	Pb, 0–40	0.1	NA	5.3	6.6	NA
FPb7	Laurentian	Pb, 0–12	0.01	NA	5.2	NA	NA
HPb5	PPHA	Pb, 0–90	0.1	NA	4.0	6.8	NA
HPb8	PUHA	Pb, 0–90	0.1	NA	5.2	NA	NA
Average		Pb		4.0	5.0	6.7	NA
S.D.				NA	0.5	0.1	NA
FNi1	Armadale	Ni, 1–6	0.1	3.8	4.6	NA	NA
FRCNi	FRC ^a	Ni, 1	0.1	NA	3.7	4.8	NA
Average		Ni		3.8	4.2	4.8	NA
S.D.				NA	0.5	NA	NA

Data are taken from compilation of Milne et al. (2003). NA = not applicable for best fit model.

^a Zhou et al., 2005.

aquatic and soil environments. Nevertheless, there are significant uncertainties inherent in applying any predictive model. Hence, our predictions should be considered qualitative, and are only approximations of the speciation in a hypothetical, ternary-free environment. Based on statistics from previous modeling exercises, uncertainties of $\pm 10\%$ are not uncommon.

3.4.3. Metal complexation in ternary experiments

The extents of metals adsorbed to *B. subtilis* in the presence of NOM (ternary systems) for Group 1 experiments are presented as open symbols for humic acid, fulvic acid, and bulk NOM in Fig. 5a–d for systems with Cd, Ni, Cu, and Pb, respectively. When adding NOM to the bacteria–metal system, two competing effects are possible: 1) aqueous metal–NOM complexation serves to diminish adsorption and 2) ternary surface complexation increases the extent of adsorption onto *B. subtilis*. The joint, ternary-free, model predicts the extents of metals bound to *B. subtilis* in the presence of NOM, ignoring ternary complexation (effect 1 only). These model predictions are presented as dashed curves in Fig. 5a–d.

The difference between the extent of metal complexation predicted by the ternary-free models (dashed curves) and the ternary experimental data (open symbols) is equal to the concentration of metal bound in

ternary form. In the Cd–bacteria–NOM systems, ternary complexes comprise approximately 20% of the Cd in the system over a pH range of ~ 7 to 9 (Fig. 5a). At lower pH (< 5.0), humic acid ternary complexes still account for $\sim 10\%$ of the total Cd, while fulvic acid and bulk NOM ternary complexes do not incorporate significant Cd (Fig. 5a). Results are similar in the Ni–bacteria–NOM systems (Fig. 5b) in that ternary complexes with all NOM fractions account for $\sim 20\%$ of the total Ni at a pH of ~ 7 to 8. Again, humic acid ternary complexes take up the most Ni at low pH, accounting for $\sim 30\%$ of the total Ni in the system below pH 5.0. Conversely, ternary complexes with fulvic acid and bulk NOM take up a relatively small percentage of the total Ni over the same pH range (Fig. 5b). The amount of Cu in ternary form is about the same above pH 5.0 for all NOM fractions (Fig. 5c). For example, $\sim 35\%$, $\sim 35\%$, and $\sim 45\%$ of the total Cu is present in ternary form at pH 6.5 in systems with fulvic acid, bulk NOM, and humic acid, respectively. About 35% of the Cu is bound in ternary form at pH 4.0 in the fulvic acid and bulk NOM systems, while humic acid ternary complexes take up $\sim 65\%$ of the total Cu at the same pH. The amount of Pb bound in ternary form appears to be more dependent upon the NOM fraction. For example, at pH 6.5 bulk NOM takes up $\sim 25\%$ of the total Pb as a

ternary complex, while only ~15% and ~0% of the total Pb is taken up in fulvic acid and humic acid ternary complexes, respectively (Fig. 5d). At pH ~4.0, humic acid ternary complexes take up ~25% of the total Pb, while fulvic acid and bulk NOM ternary complexes take up ~15% and ~0%, of total Pb, respectively.

The extents of metals adsorbed to *B. subtilis* in the presence of humic acid for Group 2 experiments are presented for systems with Cd, Ni, Cu, and Pb Fig. 6. The predicted extents of metal adsorption in this system, neglecting the formation of ternary complexes, are presented as dashed curves with symbols that correspond with the symbols for each of the respective metals (Fig. 6). The results demonstrate that when compared at identical molar component ratios, Cu is incorporated into ternary complexes to the greatest extent and Cd to the least extent. Both Pb and Cu are incorporated into humic ternary complexes to greater extents as pH decreases, while Cd and Ni humic ternary complexes decrease in concentration as pH decreases.

The incorporation of metals into ternary complexes for Group 2 experiments is largely opposite of what was observed for the incorporation of humic acid into ternary complexes. The Ni and Cd experimental systems facilitate the incorporation of greater amounts of humic acid into ternary complexes than systems with Pb and Cu (Fig. 3). Oppositely, as pH decreases, the metals themselves (Cd and Ni) are incorporated into ternary complexes to lesser extents than Pb and Cu (Fig. 6). This metal behavior can partly be explained by the fact that Pb and Cu form stronger binary complexes with bacteria and NOM than do Ni and Cd (see Fein et al., 2001; Milne et al., 2003, for examples). Hence, we would expect that more Pb and Cu would be bound to both bacteria and NOM in binary or ternary form as compared to Ni or Cd. However, this does not explain the observation that more humic acid is incorporated in Ni and Cd ternary complexes over the entire pH range tested (Fig. 3). We speculate that this may also be attributable to differences in the respective bonding strengths. Ni and Cd are more likely to form weaker, possibly outer sphere, complexes with bacteria and NOM. These weaker bonds may cause Ni and Cd to preferentially act as bridging cations for NOM and bacteria, resulting in the observed increase of humic acid in ternary form. Oppositely, Pb and Cu are more likely to remain tightly bound to the individual components, causing less NOM to be incorporated per quantity of metal involved in each ternary complex. Additional explanation is required as to why negligible quantities of Pb are incorporated into ternary complexes at pH values >5.0. We further address this issue below.

3.5. Ternary reactions

The stoichiometry of ternary complexation reactions can change as a function of pH and metal:NOM:bacteria concentration ratios, making ternary reactions difficult to model. Furthermore, for ternary complexes that involve large molecules such as NOM, writing site-specific reactions may not represent chemical reality. For example, each NOM molecule bound to the bacterial surface may sterically block an unknown quantity of bacterial surface functional group sites, effectively decreasing their activities and decreasing their abilities to bind metals or other organic molecules. In addition, each adsorbed NOM molecule possesses a number of functional group sites that are not involved directly with binding onto the cell wall, and it is not known how many of these sites will be active or accessible for metal and/or surface complexation once the NOM is associated with the bacterial surface as a ternary complex (e.g., these sites may become blocked). Because of these complexities, the first step in modeling ternary complexation is to ascertain whether the stoichiometries of the important ternary complexation reactions change under different experimental conditions. We track the average reaction stoichiometry for ternary complexation as function of pH and metal:NOM:bacteria concentration ratios based on the extent of NOM and metal removal from solution due to ternary complexation.

In order to evaluate ternary stoichiometries under comparable component concentrations, we initially focused on Group 2 experiments (Figs. 3 and 6). Estimates for the concentrations of metals and humic acid removed as ternary complexes were made every 0.5 pH units from pH 4 to 7 (pH 7 data were not available for the Cu and Pb systems). The percentage of metal involved in ternary complexation could be determined directly and converted to molar concentration (see above), and the percentage of humic acid involved in ternary complexation was converted to molar concentration units based on the average site concentration of 4.9×10^{-3} mol of humic sites/g, as determined by Borrok and Fein (2005) using titration data from a variety of aquatic and soil NOM fractions. The molar humic:metal ratio involved in ternary complexation was then normalized to the molar concentration of bacterial surface sites present in each experiment (3.1×10^{-4} mol of bacteria sites/g, Fein et al., 2005). This normalized humic:metal ratio, therefore, reflects the stoichiometric molar proportions of NOM sites, metal, and bacterial surface sites involved in each ternary complex. The normalized humic:metal ratios for Group 2 experiments are presented in Fig. 7. Humic:metal ratios for Cu, Cd, and Ni ternary complexes increase with

decreasing pH possibly due to an increase in hydrophobic attraction between bacteria and NOM with decreasing pH that leads to enhanced NOM adsorption under low pH conditions. Moreover, Cu exhibits the lowest humic:metal ratio over the entire pH range, followed by Ni and then Cd. This stoichiometric relationship demonstrates that Cu ternary complexes involve less NOM per mole of adsorbed metal than do Ni or Cd ternary complexes. This is almost certainly attributable to the relative binding strength of these metal cations with the bacterial surface and NOM. For example, the order $Cd < Ni < Cu$ reflects the weakest to strongest binding strengths for these metals with analog carboxyl ligands such as oxalate and citrate (Smith and Martell, 1987). We speculate that metals that form weaker chemical bonds with the bacterial surface and NOM are more efficient at forming electrostatic bridges between negatively charged NOM and bacteria (e.g., resulting in higher NOM concentrations per metal). Oppositely, metals such as Cu, with extremely high binding affinities, bind so tightly to the individual components that ternary complexes are less likely to involve large concentrations of NOM. However, because Cu binds so strongly to NOM and bacteria, Cu-bearing ternary complexes still take up considerable quantities of total Cu, regardless of how much NOM is present (see Fig. 6). Conversely, Ni- and Cd-bearing ternary complexes are comparatively ‘metal deficient’.

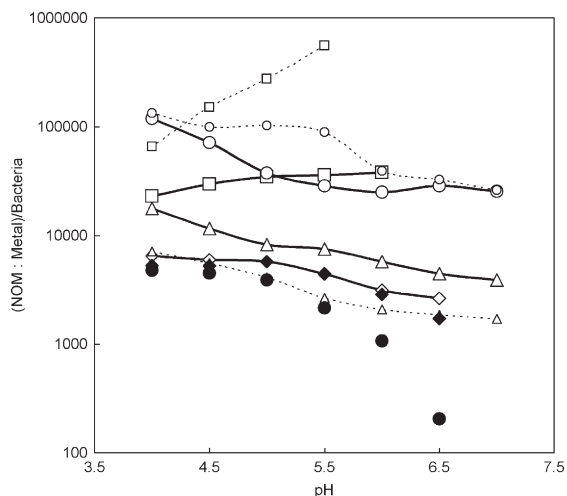


Fig. 7. Estimated molar ratios of [‘NOM sites’ to ‘metal’ normalized to ‘bacterial surface sites’] for ternary complexes as a function of pH. Results are presented for Group 2 experiments as solid curves connecting symbols for Cu (\diamond), Pb (\square), Ni (Δ), and Cd (\circ), and for Group 1 experiments (involving humic acid) as dashed curves connecting symbols for Pb (\square), Ni (Δ), and Cd (\circ). Solid symbols represent Cu-bearing ternary complexes with fulvic acid (\bullet) and bulk NOM (\blacklozenge).

The behavior of the normalized humic acid:Pb ratio for Group 2 ternary complexes as a function of pH is opposite to that observed for Cu, Ni, and Cd. The humic acid:Pb ratio of Pb-bearing ternary complexes decreases slightly with decreasing pH. It is possible that this effect is caused by elevated concentrations of binary Pb–NOM complexes under high pH conditions that serve to keep more Pb from forming ternary complexes with the bacterial surface. This may help to explain why very little Pb is involved in ternary complexation above pH 5.0 (Fig. 6). However, despite low ternary Pb involvement, it appears that humic acid is still attracted to the bacterial surface to some extent over the entire pH range tested (Fig. 3).

Normalized humic:metal ratios were also calculated for Group 1 experiments, which were conducted using different sets of component concentrations. Here we focus only on experiments involving humic acid to eliminate the type of NOM fraction as a variable. The humic:metal ratios for ternary complexes in Group 1 experiments are presented as dashed curves in Fig. 7 (note that Cu experiments were conducted under a single set of component concentrations, and therefore no additional Cu–humic acid results are presented). The humic:Cd and humic:Ni ratios for Group 1 experimental conditions are close to those calculated for Group 2 experiments, displaying the same trend of increasing NOM:metal ratio with decreasing pH. The humic:Pb ratio increases with increasing pH like those calculated for Group 2 experiments, although the magnitude of the humic:Pb ratios for Group 1 and Group 2 experiments are considerably different. These results reinforce the previous observations regarding ternary stoichiometries and also demonstrate that the stoichiometries of the important ternary surface complexes are not constant as a function of pH or component concentrations.

In addition to being affected by pH and component concentration, the ternary reaction stoichiometry is also affected by the type of NOM fraction present in solution. Fig. 7 also depicts the normalized fulvic acid:Cu and bulk NOM:Cu ratios as solid symbols for comparison. These experiments were conducted under identical component concentrations to the ternary experiments involving Cu and humic acid. While the bulk NOM:Cu ratios are in good agreement with the observed humic:Cu ratios experiments, the fulvic:Cu ratios are significantly different, particularly so at higher pH. This comparison further demonstrates that ternary reaction stoichiometries are dependent upon a variety of factors, including the type of NOM molecules involved. Hence, it is unreasonable to expect that ternary complexes can be quantified using thermodynamic chemical equilibrium models with a single average stoichiometry and

corresponding stability constant, except if a narrow range of conditions is considered. Without a molecular-scale understanding of the ternary formation process and information on the types of metals and NOM molecules involved, predictions of the extents of ternary complexation appear to be limited to the specific conditions approximated in laboratory experiments.

4. Conclusions

The results of this investigation suggest that ternary, bacteria–metal–NOM complexes are present under the conditions found in many natural aquatic settings, and that their formation is a rapid, fully reversible chemical process (Fig. 4). pH has the greatest impact on the extent, and therefore significance, of bacteria–metal–NOM complexation. All NOM fractions form greater amounts of ternary complexes as solution pH decreases, presumably due to the control of hydrophobic forces (e.g. Figs. 2a–d and 3). However, metal incorporation into ternary complexes is more complicated, and is highly dependent upon the identity of the metal involved. Ternary complexes with Cu incorporate the least amount of NOM per amount of metal, while ternary complexes with Cd incorporate the greatest amount of NOM per amount of metal (Fig. 7). We speculate that this behavior may be a function of the relative bonding strengths of these metals with organic ligands, with the elements that form the weakest bonds to organic ligands leading to the most extensive formation of ternary complexes. This implies that even small concentrations of elements like Ca and Mg that have relatively weak affinities for bacterial surfaces and NOM are still likely to facilitate the formation of bacteria–metal–NOM complexes. The NOM:metal ratio of ternary complexes is also pH dependent. The NOM:metal ratio for Cu, Ni, and Cd ternary complexes increases with decreasing pH, presumably due to increased hydrophobic attraction of NOM at low pH (Fig. 7). However, the NOM:metal ratio decreases as pH decreases for ternary complexes with Pb. Humic acid ternary complexes incorporate very little Pb at pH values >5.0 (Fig. 6), but still manage to involve significant amounts of humic acid (Fig. 3).

The character of the organic molecules tested (humic acid, fulvic acid, or bulk NOM) also impacts the formation of bacteria–metal–NOM complexes. Under acidic pH conditions (≤ 4.0) the humic acid fraction is always incorporated into ternary complexes to the greatest extent (Fig. 2). Because the humic acid fraction contains high molecular weight, aromatic molecules, the observed increase in humic acid ternary complexation over other NOM fractions is likely attributable to greater

hydrophobic interactions. Moreover, the NOM:metal ratios can be significantly different for ternary complexes involving humic and fulvic acids in otherwise comparable conditions (Fig. 7).

These results suggest that the mobility of metals in waters of neutral pH can be significantly impacted by ternary complexation among bacteria, metals, and NOM. For example, ternary complexation can increase the amount of Cu and Ni bound to the bacterial surface by 35 to 40% at neutral pH (Fig. 6), effectively lowering the concentrations of dissolved binary NOM–metal complexes and increasing the concentrations of colloidal bacteria–metal–NOM complexes. Unfortunately, bacteria–metal–NOM interactions present significant modeling difficulties because they are dynamic, changing as a function of component concentrations, pH, and the types of NOM molecules and metals involved. Until a molecular understanding of these reactions is achieved, it appears that the only sure method for determining the effect of ternary complexation in real systems is to test each system of interest individually.

Acknowledgments

Research funding was provided by the National Science Foundation through grants EAR99-05704, EAR02-07169, and EAR02-21966. D.B. was partially supported through a University of Notre Dame Arthur J. Schmitt Fellowship. Thanks to Patricia Maurice for providing XAD-8 resin material. The manuscript was significantly improved through insightful comments by two anonymous journal reviewers.

References

- Aiken, G.R., 1985. Isolation and characterization techniques for aquatic humic substances. In: Aiken, G.R., McKnight, D.M., Wershaw, R.L., MacCarthy, P. (Eds.), *Humic Substances in Soil, Sediment, and Water*. Wiley, New York, pp. 363–385.
- Benedetti, M.F., van Riemsdijk, W.H., Koopal, L.K., Kinniburgh, D.G., Goody, D.C., Milne, C.J., 1996. Metal ion binding by natural organic matter: from the model to the field. *Geochim. Cosmochim. Acta* 60, 2503–2513.
- Beveridge, T.J., 1989. Role of cellular design in bacterial metal accumulation and mineralization. *Annu. Rev. Microbiol.* 43, 147–171.
- Beveridge, T.J., Fyfe, W.W., 1985. Metal fixation by bacterial-cell walls. *Can. J. Earth Sci.* 22, 1893–1898.
- Beveridge, T.J., Murray, R.G.E., 1980. Sites of metal-deposition in the cell-wall of *Bacillus subtilis*. *J. Bacteriol.* 141, 876–887.
- Borrok, D., Fein, J.B., 2004. Distribution of protons and Cd between bacterial surfaces and dissolved humic substances determined through chemical equilibrium modeling. *Geochim. Cosmochim. Acta* 68, 3043–3053.

- Borrok, D.M., Fein, J.B., 2005. The impact of ionic strength on the adsorption of protons, Pb, Cd, and Sr onto the surfaces of Gram negative bacteria: testing non-electrostatic, diffuse, and triple-layer models. *J. Colloid Interface Sci.* 286, 110–126.
- Borrok, D., Fein, J.B., Kulpa, C.F., 2004a. Proton and Cd adsorption onto natural bacterial consortia: testing universal adsorption behavior. *Geochim. Cosmochim. Acta* 68, 3231–3238.
- Borrok, D., Fein, J.B., Tischler, M., O'Loughlin, E., Meyer, H., Liss, M., Kemner, K.M., 2004b. The effect of acidic solutions and growth conditions on the adsorptive properties of bacterial surfaces. *Chem. Geol.* 209, 107–119.
- Borrok, D., Turner, B.F., Fein, J.B., 2005. A universal surface complexation framework for modeling proton binding onto bacterial surfaces in geologic settings. *Am. J. Sci.* 308, 826–853.
- Brady, B., Pagenkopf, G.K., 1978. Cadmium complexation by soil fulvic acid. *Can. J. Chem.* 56, 2331–2336.
- Browne, B.A., Driscoll, C.T., 1993. pH-dependent binding of aluminum by a fulvic acid. *Environ. Sci. Technol.* 27, 915–922.
- Cabaniss, S.E., Shuman, M.S., 1988. Copper binding by dissolved organic matter: I. Suwannee river fulvic acid equilibria. *Geochim. Cosmochim. Acta* 52, 185–193.
- Carlson, G., Silverstein, J., 1997. Effect of ozonation on sorption of natural organic matter by biofilm. *Water Res.* 31, 2467–2478.
- Daughney, C.J., Fein, J.B., Yee, N., 1998. A comparison of the thermodynamics of metal adsorption onto two common bacteria. *Chem. Geol.* 144, 161–176.
- Esparza-Soto, M., Westerhoff, P., 2003. Biosorption of humic and fulvic acids to live activated sludge biomass. *Water Res.* 37, 2301–2310.
- Espisito, A., Pagnanelli, F., Lodi, A., Solisio, C., Veglio, F., 2001. Biosorption of heavy metals by *Sphaerotilus natans*: an equilibrium study at different pH and biomass concentrations. *Hydrometallurgy* 60, 129–141.
- Fein, J.B., 2000. Quantifying the effects of bacteria on adsorption reaction in water–rock systems. *Chem. Geol.* 169, 265–280.
- Fein, J.B., 2002. The effects of ternary surface complexes on the adsorption of metal cations and organic acids onto mineral surfaces. In: Hellmann, R., Wood, S.A. (Eds.), *Water–Rock Interactions, Ore Deposits, and Environmental Geochemistry: A Tribute to David A. Crerar*. Geochemical Society Special Pub., vol. 7. 462 pp.
- Fein, J.B., 2006. Thermodynamic modeling of metal adsorption onto bacterial cell walls: current challenges. *Adv. Agron.* 90, 179–202.
- Fein, J.B., Daughney, C.J., Yee, N., Davis, T.A., 1997. A chemical equilibrium model for metal adsorption onto bacterial surfaces. *Geochim. Cosmochim. Acta* 61, 3319–3328.
- Fein, J.B., Boily, J.F., Güçlü, K., Kaulbach, E., 1999. Experimental study of humic acid adsorption onto bacteria and Al-oxide mineral surfaces. *Chem. Geol.* 162, 33–45.
- Fein, J.B., Martin, A.M., Wightman, P.G., 2001. Metal adsorption onto bacterial surfaces: development of a predictive approach. *Geochim. Cosmochim. Acta* 65, 4267–4273.
- Fein, J.B., Boily, J.F., Yee, N., Gorman-Lewis, D., Turner, B.F., 2005. Potentiometric titrations of *Bacillus subtilis* cells to low pH and a comparison of modeling approaches. *Geochim. Cosmochim. Acta* 69, 1123–1132.
- Fowle, D.A., Fein, J.B., 2000. Experimental measurements of the reversibility of metal–bacteria adsorption reactions. *Chem. Geol.* 168, 27–36.
- Frost, P.C., Maurice, P.A., Fein, J.B., 2003. The effect of cadmium on fulvic acid adsorption to *Bacillus subtilis*. *Chem. Geol.* 200, 217–224.
- Herbelin, A.L., Westall, J.C., 1999. FITEQL, a computer program for determination of chemical equilibrium constants from experimental data. Report 99-01 — Dept. of Chem., Oregon State Univ. Corvallis, OR, USA.
- Harvey, R.W., Voss, C.I., Souza, W.R., 1989. Comparison of bacterial and solute transport through structured and unstructured porous media. *Ground Water* 27, 720.
- Haas, J.R., 2001. Thermodynamics of U(VI) sorption onto *Shewanella putrefaciens*. *Chem. Geol.* 180, 33–54.
- He, L.M., Tebo, B.M., 1998. Surface charge properties of and Cu(II) adsorption by spores of the marine *Bacillus* sp. Strain SG-1. *Appl. Environ. Microbiol.* 64, 1123–1129.
- Jin, X., Bailey, G.W., Yu, Y.S., Lynch, A.T., 1996. Kinetics of single and multiple metal ion sorption processes on humic substances. *Soil Sci.* 161, 509–520.
- Johnson, W.P., Logan, B.E., 1996. Enhanced transport of bacteria in porous media by sediment-phase and aqueous-phase natural organic matter. *Water Res.* 30, 923–931.
- Kappler, A., Benz, M., Schink, B., Brune, A., 2004. Electron shuttling via humic acids in microbial iron (III) reduction in a freshwater sediment. *FEMS Microbiol. Ecol.* 47, 85–92.
- Kulczycki, E., Ferris, F.G., Fortin, D., 2002. Impact of cell wall structure on the behavior of bacterial cells as sorbents of cadmium and lead. *Geomicrobiol. J.* 19, 553–565.
- Ledin, M., Drantz-Rulcker, C., Allard, R., 1996. Zn, Cd, and Hg accumulation by microorganisms. Organic and inorganic solid components in multi-compartment systems. *Soil Biol. Biochem.* 28, 791–799.
- Ma, H., Kim, S.D., Cha, D.K., Allen, H.E., 1999. Effect of kinetics of complexation by humic acid on toxicity of copper to *Ceriodaphnia dubia*. *Environ. Toxicol. Chem.* 18, 828–837.
- Malard, F., Reygrobelle, J.L., Soulie, M., 1994. Transport and retention of fecal bacteria at sewage-polluted fractured rock sites. *J. Environ. Qual.* 23, 1352–1363.
- Maurice, P.A., Pullin, M.J., Cabaniss, S.E., Zhou, Q., Namjesnik-Dejanovic, K., Aiken, G.R., 2002. A comparison of surface water natural organic matter in raw filtered samples, XAD, and reverse osmosis isolates. *Water Res.* 36, 2357–2371.
- Maurice, P.A., Manecki, M., Fein, J.B., Schaefer, J., 2004. Fractionation of an aquatic fulvic acid upon adsorption to the bacterium, *Bacillus subtilis*. *Geomicrobiol. J.* 21, 69–78.
- Milne, C.J., Kinniburgh, D.G., Van Riemsdijk, W.H., Tipping, E., 2003. Generic NICA–Donnan model parameters for metal-ion binding by humic substances. *Environ. Sci. Technol.* 37, 958–971.
- Ngwenya, B.T., Sutherland, I.W., Kennedy, L., 2003. Comparison of the acid–base behavior and metal adsorption characteristics of a gram-negative bacterium with other strains. *Appl. Geochem.* 18, 527–538.
- Plette, C.C., Benedetti, M.F., Van Riemsdijk, W.H., Van der Wal, A., 1995. pH dependent charging behavior of isolated cell walls of a gram-positive soil bacterium. *J. Colloid Interface Sci.* 173, 354–363.
- Richards, J.G., Curtis, P.J., Burnison, B.K., Playle, R.C., 2001. Effects of natural organic matter source on reducing metal toxicity to rainbow trout (*Oncorhynchus mykiss*) and on metal binding to their gills. *Environ. Toxicol. Chem.* 20, 1159–1166.
- Ritchie, J.D., Perdue, E.M., 2003. Proton-binding study of standard and reference fulvic acids, humic acids, and natural organic matter. *Geochim. Cosmochim. Acta* 67, 85–96.
- Royer, R.A., Burgos, W.D., Fisher, A.S., Unz, R.F., Dempsey, A.A., 2002. Enhancement of biological reduction of hematite by electron shuttling and Fe(II) complexation. *Environ. Sci. Technol.* 36, 1939–1946.
- Schindler, P.W., 1990. Co-adsorption of metal ions and organic ligands; formation of ternary surface complexes. In: Hochella Jr.,

- M., White, A.F. (Eds.), Mineral–Water Interface Geochemistry. Reviews in Mineralogy, vol. 23. Min. Soc. America, pp. 281–307.
- Schmitt, D., Saravia, F., Frimmel, F.H., Schussler, W., 2003. NOM-facilitated transport of metal ions in aquifers: importance of complex-dissociation kinetics and colloid formation. *Water Res.* 37, 3541–3550.
- Small, T.D., Warren, L.A., Roden, E.E., Ferris, F.G., 1999. Sorption of strontium by bacteria, Fe(III) oxide, and bacteria–Fe(III) oxide composites. *Environ. Sci. Technol.* 33, 4465–4470.
- Smith, R.M., Martell, A.E., 1987. *Critical Stability Constants*, vol. 4. Plenum Press, New York.
- Sokolov, I., Smith, D.S., Henderson, G.S., Gorby, Y.A., Ferris, F.G., 2001. Cell surface electrochemical heterogeneity of the Fe(III)-reducing bacteria *Shewanella putrefaciens*. *Environ. Sci. Technol.* 35, 341–347.
- Tipping, E., Rey-Castro, C., Bryan, S.E., Hamilton-Taylor, J., 2002. Al (III) and Fe(III) binding by humic substances in freshwaters, and implications for trace metal speciation. *Geochim. Cosmochim. Acta* 66, 3211–3224.
- Tornabene, T.G., Edwards, H.W., 1972. Microbial uptake of lead. *Science* 176, 1334–1335.
- Tortell, P.D., Maldonado, M.T., Granger, J., Price, N.M., 1999. Marine bacteria and biogeochemical cycling of iron in the oceans. *FEMS Microbiol. Ecol.* 29, 1–11.
- Van der Wal, A., Norde, W., Zehnder, A.J.B., Lyklema, J., 1997. Determination of the total charge in the cell walls of gram-positive bacteria: colloids and surf. *B. Biointerfaces* 9, 81–100.
- Westall, J.C., Jones, J.D., Turner, G.D., Zachara, J.M., 1995. Models for association of metal ions with heterogeneous environmental sorbents. 1. complexation of Co(II) by leonardite humic acid as a function of pH and NaClO₄ concentration. *Environ. Sci. Technol.* 24, 951–960.
- Wightman, P.G., Fein, J.B., 2001. Ternary interaction in a humic acid–Cd–bacteria system. *Chem. Geol.* 180, 55–65.
- Xue, H., Sigg, L., 1999. Comparison of the complexation of Cu and Cd by humic or fulvic acids and by ligands observed in lake waters. *Aquat. Chem.* 5, 313–335.
- Yee, N., Fein, J.B., 2001. Cd adsorption onto bacterial surfaces: a universal adsorption edge? *Geochim. Cosmochim. Acta* 65, 2037–2042.
- Yee, N., Fowle, D.A., Ferris, F.G., 2004. A Donnan potential model for metal sorption onto *Bacillus subtilis*. *Geochim. Cosmochim. Acta* 68, 3657–3664.
- Zhou, P., Yan, H., Baohua, G., 2005. Competitive complexation of metal ions with humic substances. *Chemosphere* 58, 1327–1337.