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Proton and Cd adsorption onto natural bacterial consortia: testing universal adsorption behavior

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Abstract—Bacterial surface adsorption can control metal distributions in some natural systems, yet it is unclear whether natural bacterial consortia differ in their adsorption behaviors. In this study, we conduct potentiometric titration and metal adsorption experiments to measure proton and Cd adsorption onto a range of bacterial consortia. We model the experimental data using a surface complexation approach to determine thermodynamic stability constants. Our results indicate that these consortia adsorb similar extents of protons and Cd and that the adsorption onto all of the consortia can be modeled using a single set of stability constants. Consortia of bacteria cultured from natural environments also adsorb metals to lesser extents than individual strains of laboratory-cultivated species. This study suggests that a wide range of bacterial species exhibit similar adsorption behaviors, potentially simplifying the task of modeling the distribution and speciation of metals in bacteria-bearing natural systems. Current models for bacteria-metal adsorption that rely on pure strains of laboratory-cultivated species likely overpredict the amount of bacteria-metal adsorption in natural systems. *Copyright © 2004 Elsevier Ltd*

1. INTRODUCTION

Bacteria are present in a wide range of environments, and the adsorption of metals onto bacterial surfaces can control the speciation and distribution of metals in many aquatic and near-surface systems (Ledin et al., 1996; Barns and Nierzwicki-Bauer, 1997; Ledin et al., 1999). Accurate and quantitative models that describe bacteria-metal adsorption are critical to understanding the availability and cycling of metals (Tornabene and Edwards, 1972; Tortell et al., 1999), for predicting the behavior of heavy metal contaminants (Volesky, 2001) and in the development of a number of contaminant remediation strategies (Macaskie and Basnakova et al., 1998; Espisito et al., 2001). Surface complexation models (SCMs), originally developed to describe the adsorption of metal ions onto mineral surfaces, have recently been applied to describe the adsorption of metals onto bacterial surfaces (Xue et al., 1988; Plette et al., 1995; Plette et al., 1996; Fein et al., 1997; He and Tebo, 1998; Fein, 2000). These models use a thermodynamic approach to describe metal adsorption onto bacterial surface functional groups through a series of mass action and mass balance equations.

One of the most problematic obstacles to the application of surface complexation modeling to realistic systems is that a given bacteria-bearing natural system can contain dozens of different bacterial species, and the number of species of environmental interest is huge and undetermined. If bacterial surfaces are unique and if each species exhibits unique adsorption properties, then it would be a Herculean task to determine the binding site concentrations and binding constants for each bacterial species of environmental interest. Experimentation on dozens of different bacterial species would be necessary just to describe the metal behavior in a single environment.

A potential solution to this dilemma arises from the recent observations that a number of bacterial species exhibit similar extents of metal adsorption (and similar proton and metal binding constants), as determined in laboratory experiments using individual pure strains of bacteria (Daughney et al., 1998; Small et al., 1999; Yee and Fein, 2001; Kulczycki et al., 2002; Ngwenya et al., 2003) and artificial mixtures of pure strains of bacteria (Yee and Fein, 2003). In this study, we test the hypothesis that consortia of bacteria originating from a wide range of soil and aquatic environments adsorb metals to similar extents, and that the adsorption behavior of all the consortia can be described with a single set of 'universal' thermodynamic stability constants rather than individual stability constants for each species present.

2. MATERIAL AND METHODS

2.1. Sampling and Growth of Bacteria

Soil and/or water samples were collected from Northern Indiana and Southern Michigan. Sample locations included a forest, a natural wetland, a river, and a wastewater treatment facility. All materials used for sample collection, including glass jars, lids, and digging scoops were sterilized and sealed in plastic bags before use. Soil samples were collected by removing the top 3 to 5 inches of topsoil and debris and then directly scooping a soil specimen using a glass sample jar. Water samples were collected by dipping the sample jar directly into the water. In the case of the wetland water sample, water was collected from a near-surface piezometer using disposable plastic tubing. Immediately after collection, sample lids were loosely placed over the jars to allow for aerobic conditions while preventing contamination. Control growth experiments were completed by briefly opening jars of sterilized growth media near sample locations and exposing them to the atmosphere. Lack of bacterial growth in the control samples suggests that the bacterial consortia we produced were most likely not contaminated during handling with species not present in the original sample.

Approximately 10 g of soil or 10 mL of water from the samples were used to inoculate the chosen broth solution. Bacteria were grown using either trypticase soy broth (TSB) with 0.5% yeast extract or soil broth (SB). SB was made with 250 mL soil extract, 2 g glucose, 1 g yeast extract, and deionized (DI) water (to 1.0 L). Soil extract was made by

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Table 1. Sample growth information.

Matrix	Location	Date Collected	Growth Media	Number of Inoculations	Duration of Growth for Each Inoculation (Days)
Soil	St. Mary's Forest #1 N. Indiana	1/8/03	SB	3	7
Water	Wastewater Treatment Plant (Final Clarifier) N. Indiana	2/5/03	TSB	1	3
			SB	1	8
Soil	St. Mary's Forest #2 N. Indiana	2/4/03	SB	2	6
Water	St. Joseph River N. Indiana	2/16/03	SB	1	8
Water	Wetland S. Michigan	3/8/03	TSB	1	3
			SB	1	7
Soil	Wetland S. Michigan	3/8/03	SB	2	6

TSB = Trypticase Soy Broth; SB = Soil Broth.

autoclaving 500 g of soil with 1 L of DI water for 1 h at 15 psi/121°C and decanting the fluid product using Whatman No. 2 filter paper. Inoculated broth solutions were gently shaken at room temperature until they were harvested, or a portion was used for reinoculation. Approximately 10 mL of the initial bacteria-broth suspension was used to inoculate larger quantities of identical broth solutions to dilute out the solid fraction present from soil samples. The number of reinoculations before harvest, the broth mixture used, and the growth duration for each consortium are presented in Table 1.

Because a particular set of growth conditions will support growth of only particular types of bacterial species, the bacteria grown for our experiments probably included only a subset of the total bacterial population present in each environment sampled. Experiments have shown that many of the individual bacterial species within natural consortia cannot survive repeated inoculations in laboratory growth media (Kaeberlein et al., 2002). However, by growing the bacteria directly in broth solutions and limiting the number of reinoculations, we hoped to achieve a range of diverse consortia that is at least representative of the diversity which exists in nature. Multiple experiments using the same samples, but grown from different media (with different growth durations), were completed to avoid some of the inherent bias in culturing the bacteria (Table 1); however, all growth conditions were aerobic, so all anaerobes were eliminated through the growth procedures. As a preliminary indication of diversity, Gram staining was conducted on most of the consortia used in the experiments. Diversity was more precisely determined through denaturing gradient gel electrophoresis (DGGE).

2.2. Freezing of Bacteria

Before each reinoculation and/or harvest for experimentation, bacterial cells suspended in their broth solutions were frozen for future DNA extraction and/or reuse. Cells frozen for reuse were suspended in a mixture of 10% sterilized glycerol and flash frozen with liquid nitrogen before storage in a −80°C freezer. Cells frozen for DNA extraction were not mixed with additional agents and were placed directly into the −80°C freezer.

2.3. Potentiometric Titration and Cd Adsorption Experiments

Bacteria were harvested from the growth media by centrifugation, transferred to test tubes, and washed five times in 0.1M $NaClO_4$ (the same electrolyte used in the experiments). Sodium perchlorate was chosen as the experimental electrolyte because perchlorate does not form complexes to an appreciable extent with protons or Cd under the experimental conditions. During each wash, the bacteria were suspended in fresh electrolyte solution using a vortex machine and stir rod. 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. 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. After 1 h, the weight of the moist bacterial pellet was determined. The weight recorded at this stage and reported here and throughout this paper is the wet weight. Previous work in our laboratory has shown that this wet weight is a reproducible quantity, but changes over a modest range depending upon the bacterial species weighed. The wet weight to dry weight ratio ranged from 3.1:1 to 6.5:1 and averaged 5:1 in individual experiments using *Pseudomonas fluorescens*, *Shewenella oneidensis*, *Staphyloccocs aureus*, *Bacillus subtilis*, and *Bacillus cereus*. The bacteria pellet was immediately used in potentiometric titrations or in Cd adsorption experiments. Although the bacterial cells remain viable after this treatment (Fein et al., 1997), they are not expected to be metabolizing during experiments because of the lack of nutrients and short (<3 h) experimentation times.

In each metal adsorption experiment, 10g/L of a bacterial consortium was suspended in a pH-neutralized stock solution of 0.1 mol/L $NaClO_4$ and 10 ppm Cd. After an initial 10 min equilibration time, the bacterial stock solution was divided into individual reaction vessels. The pH of each reaction vessel was adjusted by adding minute aliquots of 1.0 mol/L or 0.1 mol/L HNO_3 or NaOH. After adjustment of the pH, and an additional 2 h of reaction time on a rotating rack, the final (equilibrium) pH of each vessel was measured. Previous studies in our laboratory have demonstrated that equilibrium of the adsorption reaction generally occurs in less than 1 h and that the adsorption reaction is fully reversible (see Fowle and Fein (2000) for a complete discussion). The individual vessels were then centrifuged and the resultant supernatant filtered (0.45 μm). The filtered supernatant was analyzed for Cd using an inductively coupled plasma-atomic emission spectroscopy technique with matrix-matched standards. The concentration of metal adsorbed to bacteria in each vessel was calculated by subtracting the concentration of metal that remained in solution (supernatant) from the original 10 ppm in the stock solution.

Cells used in titration experiments were unfrozen from the frozen stock and grown in the same broth solution used for the metal adsorption experiments. Titrations were not completed on all consortia for all growth conditions, but were completed for a single consortium from each environment using one growth medium. Cells frozen from the next to final inoculation used in the metal adsorption experiments were unfrozen and inoculated/cultured for use in the titration experiments. Hence, the final growth times and number of inoculations remained the same for each consortia for each type of experiment. Several duplicate Cd adsorption experiments (data not shown) were performed using bacterial consortia grown from the frozen stock to test if the freezing process affected the observed adsorption behavior. The results using the frozen cells were indistinguishable from those using cells before the freezing step, indicating that the freezing process had no measurable effect on the experimental results. Minor variations in the diversity of the bacterial consortia in response to the freezing process cannot be ruled out. However, for this study, we determined that potential minor variations in diversity caused by deep-freezing were preferable to the

Table 2. Relative band intensities within individual lanes of DGGE gels.

Number of Bands	[3]St. Mary's Forest #2, SB	[1]Wetland Soil, SB	[1]Wastewater Effluent, SB	[2]Wastewater Effluent, TSB	[2]River Water, SB	[2]Wetland Water, TSB
1	11%	5% ± 1.0%	2% ± 0.3%	31% ± 5.5%	3% ± 0.4%	72% ± 4.5%
2	26%	2% ± 0.6%	27% ± 0.2%	69% ± 5.5%	15% ± 0.3%	28% ± 4.5%
3	18%	15% ± 1.8%	52% ± 1.7%		18% ± 2.0%	
4	31%	11% ± 0.4%	19% ± 1.9%		12% ± 1.5%	
5	14%	22% ± 0.4%			18% ± 1.0%	
6		45% ± 3.1%			34% ± 0.5%	

The band intensity is a proxy for the abundance of a specific bacterial species within each consortium. Statistics are based upon [1]3 replicates (1 σ uncertainties); [2]2 replicates (maximum difference); or [3]no replicates were used. The bands for each consortium are unique and are not comparable to bands in other consortia. Samples displaying a single band are not shown.

TSB = Trypticase Soy Broth; SB = Soil Broth.

large changes in diversity expected from the alternatives of either growing the consortia for longer periods of time, or resampling and regrowth of the consortia before each type of experiment conducted here.

The cells were harvested from the growth media and washed as described above; however, for the titrations, they were suspended in approximately 10 mL of 0.1 mol/L $NaClO_4$ that had been purged of CO_2 by N_2 bubbling for 60 min. The suspension was immediately placed into a sealed titration vessel maintained under a positive pressure of N_2. Titrations were conducted using an automated burette assembly with aliquots of 1.001 N NaOH and/or 1.0001N HNO_3.

2.4. DGGE Analysis

DNA was extracted directly from the cell suspensions in the growth media using a MoBio Laboratories, Inc. Ultraclean soil DNA kit. Extracted DNA was frozen at −20°C before amplification. A custom-made universal bacterial primer set (EUB 341 and EUB 534, 200 base pairs in length with a GC-clamp) was used during the polymerase chain reaction (PCR) process to ensure that only the DNA extracted from bacteria would be amplified. However, this approach cannot guarantee that DNA from every bacterial species in the sample becomes amplified. DGGE was carried out using a Dcode universal mutation detection system (Bio-Rad). The PCR product was loaded into a gel with a 30 to 60% gradient of a chemical denaturant (urea and formamide). The DNA was forced to travel through the denaturant in response to an electrical potential. Gels were run at 60°C and a potential of 60 V for 14 h. The gel was then stained using ethidium bromide, fluoresced, and photographed using a Kodak EDAS 290 photographic system. Because of the differences in DNA base pair sequences among different bacterial species, the DNA from a specific species will denature at a specific point in the gel, forming a characteristic band. The intensity of these bands is directly related to the concentration of DNA present in the sample. Hence, analysis of the band positions and intensities can determine the minimum number of bacterial species present, and their relative abundances. The position and relative intensities of these bands were analyzed using Kodak ID 3.6 software. Although this is a sufficient test for diversity in our study, additional sequencing of the bands would be necessary to determine the identity of each band and to verify that each band represents only one species.

3. RESULTS AND DISCUSSION

Gram-staining revealed both gram-positive and gram-negative bacterial species in all consortia examined. DGGE results indicated that most of the consortia displayed three to six bands (species) and that there was little or no similarity of bands from one sampled environment to the next (Table 2 and Fig. 1). Two of the consortia displayed only two bands, while one forest soil consortium contained only one band. One of the wetland water samples displayed only one very faint band, indicating either that something other than bacteria dominated this consortium, or, more likely, that the DNA extracted from this sample was not successfully amplified using the PCR technique and chosen primers. Gram staining of this consortium identified numerous rod-shaped gram-negative bacteria and much fewer oval-shaped gram-positive bacteria.

All the consortia exhibited significant buffering capacity over the entire pH range studied (2.5–9.7). Representative titration data from forest soil consortium #2 are presented in Figure 2. The titration curves for all of the consortia were similar in shape to each other and to those determined previously for a wide range of individual bacterial species (Fein et al., 1997; Yee and Fein, 2001). The extent of Cd adsorption onto each bacterial consortium studied here increases with increasing pH (Fig. 3), defining a pH adsorption edge. Data collected from experiments using consortia of bacteria from each tested site form a relatively narrow band, demonstrating that the experimental consortia adsorb metals to similar extents (Fig. 3). Changing the growth media and duration of growth for individual samples had no discernable effect on the relative positions of the adsorption edges, despite the fact that the bacterial speciation within the consortia appeared to change (Table 2).

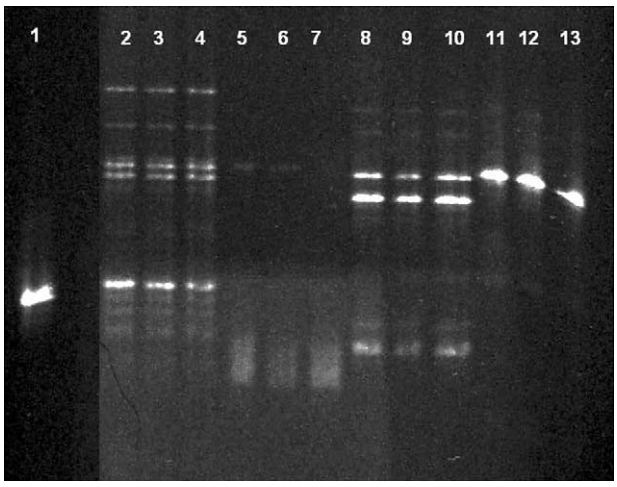

Fig. 1. Example DGGE gel with numbered lanes. 1 Control, E. Coli; 2–4 Wetland Soil, SB; 5–7 Wetland Water, SB; 8–10 Wastewater, SB; 11–13 Forest Soil #1, SB. TSB = Trypticase Soy Broth; SB = Soil Broth.

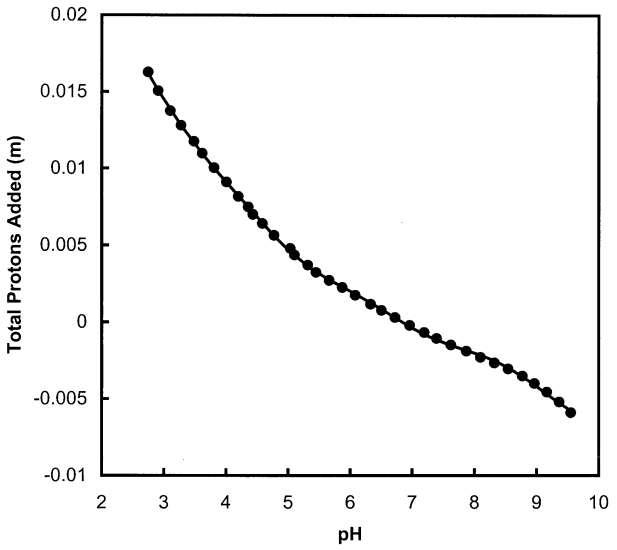

Fig. 2. Potentiometric titration data (solid circles), and best-fit model using 4 surface sites (curve), for the forest soil #2 consortium. Values are positive for net acid added; negative for net base added.

3.1. Surface Complexation Modeling

The similar adsorption behaviors that we observed for each of the consortia in this study suggest that it may be possible to model metal adsorption onto bacterial consortia with a single 'universal' set of thermodynamic properties. We model the observed adsorption behavior using a discrete site SCM approach. This model of cell wall adsorption is a simplification of the mechanisms involved in what is likely an extremely complex and heterogeneous chemical system. However, discrete site surface complexation models offer a number of advantages. For example, they are relatively simple, yet they can describe adsorption behavior over a wide range of chemical conditions, and they are relatively easy to adapt for use in many contaminant transport codes. We use the SCM approach to explicitly account for cell wall adsorption reactions, and we use the experimental data to constrain a system of mass action and mass balance equations to solve for equilibrium constants and/or concentrations of individual chemical species (Fein et al., 1997). We represent bacterial surface functional groups using a limited number of discrete site types, each of which undergoes deprotonation according to the following reaction:

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

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

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

where [R-A$^-$] and [R-AH°] represent the concentration of deprotonated and protonated sites, respectively, and a_{H^+} represents the activity of protons in the bulk solution.

The titration data were modeled to determine functional group site concentrations and acidity constant values. These parameters were calculated using the chemical equilibrium program FITEQL 2.0 (Westall, 1982). We chose to neglect the effects of the surface electric field on the adsorption reactions (nonelectrostatic model) for two reasons. Because all the experiments were conducted at a single ionic strength value (0.1 m), there is no way to uniquely constrain a type of surface electric field model that best accounts for the experimental data. Also, to implement an electrostatic model, the surface area of the bacteria used in the experiments must be calculated. Each consortium used in these experiments contained a variety of bacterial species of different shapes and sizes. Therefore, the overall surface area could not be calculated with any certainty.

In our modeling approach, we determine the minimum number of discrete functional group types that are required to account for the observed buffering capacity of each consortium by sequentially testing models with one through five proton-active sites. In each case, the four site models yield significantly better fits than models with fewer sites. The five site models in each case do not converge, indicating that the data cannot constrain a model with 5 discrete functional group types. An example model fit for the titration data generated from the forest sample #2 consortium is presented in Figure 2, and the average functional group site concentrations and acidity constant values are compiled in Table 3. We refer to the functional group sites with pK_a values of 3.12, 4.70, 6.57, and 8.99 as Sites 1 through 4, respectively. The limited chemical (Beveridge and Murray, 1980; Beveridge and Fyfe, 1985) and spectroscopic (Boyanov et al., 2002; Kelly et al., 2002) information available for pure strains of bacteria suggests that the

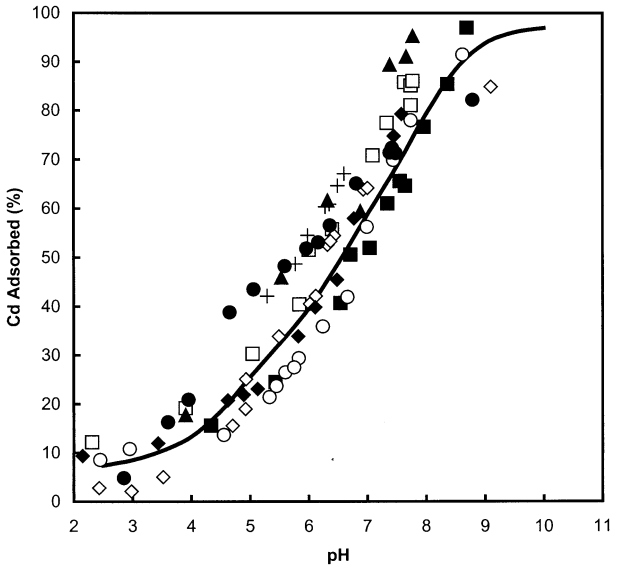

Fig. 3. Cd adsorption onto bacterial consortia cultured from soil and aquatic environments. All Cd adsorption experiments were conducted using 10 ppm Cd and 10 g/L consortia (wet weight) in a suspension of 0.1 mol/L $NaClO_4$. The consortia were grown using soil broth (SB) or trypticase soy broth (TSB) with 0.5% yeast extract. ■ = Forest Soil #1, SB; □ = Wastewater Effluent, TSB; ▲ = Wastewater Effluent, SB; ◇ = Forest Soil #2, SB; ○ = River Water, SB; ● = Wetland Water, TSB; ◆ = Wetland Soil, SB; + = Wetland Water, SB. The solid line represents the predicted adsorption edge that is calculated using the 'universal' functional group site concentrations and binding constants developed in this study.

Table 3. Site concentrations and proton binding constants (K_a), with 1 σ uncertainties, for the four functional group sites identified through triplicate potentiometric titrations.

	Proton Binding Constants ($-\log K_a$)				Site Concentrations ($\times 10^{-5}$ moles/g wet weight)			
Consortia	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4
Wetland Soil, TSB	2.99 ± 0.05	4.62 ± 0.05	6.62 ± 0.15	9.18 ± 0.08	7.06 ± 0.62	5.70 ± 0.25	2.69 ± 0.24	3.90 ± 0.46
Wastewater Effluent, TSB	3.19 ± 0.04	4.74 ± 0.10	6.46 ± 0.07	8.94 ± 0.02	8.06 ± 0.36	9.79 ± 0.37	4.96 ± 0.24	4.39 ± 0.46
Forest Soil # 2, SB	3.22 ± 0.04	4.74 ± 0.07	6.65 ± 0.02	9.11 ± 0.10	7.61 ± 0.29	6.94 ± 0.17	4.62 ± 0.26	6.56 ± 1.30
River, SB	3.09 ± 0.20	4.74 ± 0.15	6.67 ± 0.20	8.74 ± 0.27	3.34 ± 0.27	3.32 ± 0.13	1.45 ± 0.13	1.70 ± 0.17
[a]Wetland Water, TSB	3.15	4.52	6.24	8.92	8.23	10.90	6.74	8.49
Average	3.12 ± 0.13	4.70 ± 0.11	6.57 ± 0.17	8.99 ± 0.21	6.65 ± 1.96	6.78 ± 2.65	3.68 ± 1.71	4.47 ± 2.19

[a] Only one of the three titrations was performed to a low enough pH to constrain fit.

TSB = Tripticase Soy Broth; SB = Soil Broth. Constants are based on equation 2, $K_a = \frac{[R - A^-]a_{H^+}}{[R - AH^\circ]}$

majority of these sites are likely phosphoryl and carboxyl. However, additional spectroscopic information is necessary to chemically identify the sites present in each consortium.

The calculated site concentrations and acidity constants are similar for all the consortia studied, despite the diversity represented in the different consortia. The 1 σ uncertainties associated with the average negative logarithm of the acidity constant (pK_a) value for each functional group site (collected over all titrations) range from 0.21 (Site 4) to 0.11 (Site 2). Analogous 1 σ uncertainties for functional group acidity constant values that were determined from similar experiments using individual bacterial species range from 0.45 to 0.20 (Yee and Fein, 2001), suggesting that the uncertainties determined in this study may arise more due to experimental variations, rather than from real systematic variations between the different consortia tested. The 1 σ uncertainties (0.17 to 0.27 on a log scale) for each of the four functional group site concentrations are slightly greater, suggesting there may be more variability in site concentrations than acidity constants for bacteria in nature. To a good first approximation, a single set of averaged site concentrations and acidity constants can describe the buffering behavior of all the consortia studied here.

We use FITEQL 2.0, along with the average acidity constant and site concentration values derived through modeling of the titration data, to determine the cadmium binding constants from the Cd adsorption data. We determine the minimum number of types of binding sites that are required to account for the observed Cd adsorption, and solve for the values of the corresponding equilibrium constants for the adsorption reactions. The speciation of Cd in the system was modeled using the equilibrium constant values from Baes and Mesmer (1976) for aqueous Cd-hydroxide species. We tested a range of adsorption reaction stoichiometries, with Cd adsorption onto the bacterial surfaces modeled as a reaction between Cd^{+2} and a protonated (x = 1) or deprotonated (x = 0) surface site (Sites 1 to 4, both individually and in combination, were tested in each case) to form a bacterial surface complex, according to the reaction:

$$Cd^{+2} + R - AH_x^{x-1} \Leftrightarrow R - AH_x(Cd)^{x+1} \quad (3)$$

with an equilibrium constant, K, for this reaction given by:

$$K = \frac{[R - AH_x(Cd)^{x+1}]}{a_{Cd^{+2}}[R - AH_x^{x-1}]} \quad (4)$$

The modeling results are compiled in Table 4. The best-fitting adsorption model for all but one consortium involves monodentate adsorption of Cd^{+2} onto the deprotonated form of each of the four functional group sites. Adsorption experiments

Table 4. Cd metal binding constants (K) for best-fit adsorption models.

	Cd binding Constants (log K)			
Consortia	Site 1 (3.12 pK_a)	Site 2 (4.70 pK_a)	Site 3 (6.57 pK_a)	Site 4 (8.99 pK_a)
Forest Soil #1, SB	2.76	2.79	3.72	5.25
Forest Soil #2, SB	2.26	3.07	4.19	4.64
Wastewater Effluent, SB	2.75	3.45		6.00
Wastewater Effluent, TSB	3.10	2.50	4.19	5.60
Wetland Soil, SB	2.96	2.44	3.86	5.66
Wetland Water, SB	3.35	2.52	4.26	5.50
Wetland Water, TSB	2.84	3.48	4.00	4.68
River, SB	2.96	1.92	3.69	5.52
Weighted Average	2.83 ± 0.30	2.70 ± 0.47	3.95 ± 0.22	5.22 ± 0.40

Functional group sites are those identified through potentiometric titrations, and weighted averages and 1 σ errors are based on the number of data points available to constrain each constant. TSB = Tripticase Soy Broth; SB = Soil Broth. Constants are based on equation 4, $K = \frac{[R - A(Cd)^{+1}]}{a_{Cd^{+2}}[R - A^{-1}]}$

using the wastewater effluent (soil broth) consortium did not cover a large enough pH range to constrain Cd binding onto all 4 sites. In this case, the adsorption behavior could be accounted for using only 3 of the 4 surface complexes; however, the calculated values of the binding constants for these complexes are similar to those obtained from the experiments that required all four surface complexes.

'Universal' Cd adsorption constants were developed for each functional group site by averaging the binding constants obtained for each site, weighting the values based on the number of data points used to constrain each individual binding constant. The average site concentration and binding constant values yield a 'universal' SCM that predicts an adsorption edge that is in reasonable agreement with the observed Cd adsorption behavior over the entire pH range of the experiments (Fig. 3). Clearly, these constants alone are not capable of determining the distribution of Cd among the many important binding ligands (e.g., bacterial surfaces, exopolysaccharide material, mineral surfaces, dissolved organic matter, inorganic ligands, etc.) in natural systems. Additional equilibrium constants for metal complexation with other environmentally relevant ligands are necessary in order for our results to be used to estimate metal distributions in realistic bacteria-bearing systems.

The bacterial consortia that we produced in this study are not exact or complete representations of the consortia of bacteria which exist in the locations that we sampled. Although the DGGE results indicate that the consortia were quite diverse and distinct from each other, their constituent species were only a subset of the ones present in the natural systems. However, the similar Cd adsorption behavior that we observed for the bacterial consortia in this study suggests that actual consortia from a wide range of natural systems adsorb metals to a similar extent as well. We hypothesize that the stability constants determined here, in conjunction with other relevant stability constants, can be used to predict the extent of Cd adsorption onto bacterial consortia that are found in a wide range of environments. This hypothesis assumes that the bacteria/metal adsorption behavior is linear over a wide range of bacteria/metal ratios. However, the dominant adsorption mechanism may be different under extremely high or low metal loading conditions. If so, the equilibrium constants for these reactions must be determined to model the effect of bacterial adsorption on metal speciation in these systems.

Although our data indicate that a wide range of bacterial consortia exhibit similar Cd adsorption behaviors, Figure 4 illustrates that the consortia adsorb significantly less metal than do equivalent amounts of individual pure laboratory strains of bacterial species cultured under identical conditions, including *Bacillus subtilis*, *Bacillus cereus*, *Pseudomonas mendocina*, *Pseudomonas aeruginosa*, and *Pseudomonas putida* (Borrok et al., 2004). The reason for the gap in adsorption behavior between pure strains of bacteria and consortia of bacteria cultured from natural environments is uncertain; however, it may be due in part to the number of growth cycles experienced by the bacteria in nutrient-rich laboratory media. The pure strains of bacteria used for the experiments shown in Figure 4 were grown and replated on nutrient-rich media numerous times over a period of months or years, while the consortia of bacteria were grown in nutrient-rich broth solutions for only a few life cycles.

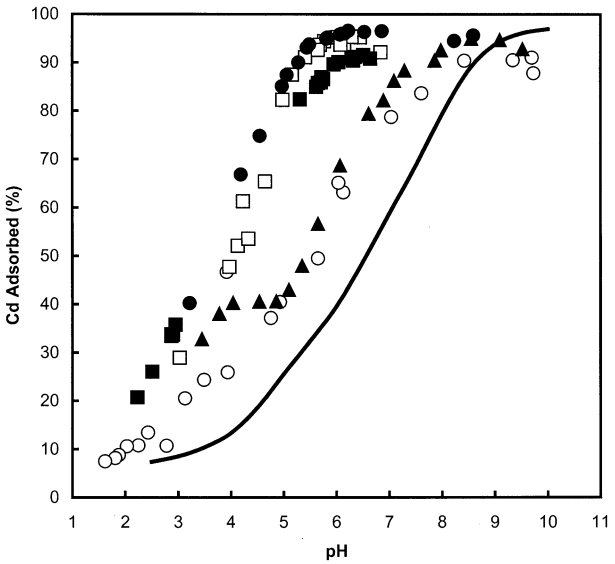

Fig. 4. Comparison of the natural adsorption edge (solid curve) to Cd adsorption data from experiments using individual, pure strains of bacteria. □ = *Pseudomonas mendocina*; ■ = *Pseudomonas putida*; ▲ = *Bacillus cereus*; ○ = *Bacillus, subtilis*; ● = *Pseudomonas aeruginosa*. All Cd adsorption experiments were conducted using 10 ppm Cd and 10 g/L consortia (wet weight) in a suspension of 0.1 mol/L $NaClO_4$. Cd adsorption onto individual bacterial species measured by Borrok et al. (2004).

The average acidity constants for the consortia from this study are nearly identical to the pK_a constants (3.3, 4.8, 6.8, and 9.1 for Sites 1 to 4, respectively) derived by J. B. Fein (personal communication) to describe proton binding onto the gram-positive bacterium, *B. subtilis*, also using a nonelectrostatic approach. However, the functional group site concentrations for *B. subtilis* (8.1×10^{-5}, 1.1×10^{-4}, 4.4×10^{-5}, and 7.4×10^{-5} moles/g wet weight for Sites 1 to 4, respectively) are higher than those for the bacterial consortia for each of the four functional group-site types. Overall, the laboratory-cultured pure strain of *B. subtilis* has ~40% more sites available for metal binding per wet gram of bacteria than the consortia tested in this study. This observation suggests that the proton binding constants for individual bacterial species and for bacterial consortia may be the same. The difference between the adsorption behaviors observed for natural consortia and for individual bacterial species appears to be caused by the lower site density associated with the bacterial consortia, rather than by a lower tendency for a proton to bind with each functional group.

4. CONCLUSIONS

This study demonstrates for the first time that diverse groups of bacteria, cultured from a variety of soil and aquatic environments, adsorb metals to similar extents. This observation could greatly simplify the task of modeling bacteria-metal adsorption in nature. Using the assumption of universality of metal adsorption behavior, along with the equilibrium constants and site concentrations determined in this study (and the relevant constants for competing metals and binding ligands), the

effects of bacterial adsorption on the distribution and speciation of Cd in realistic geologic systems can be modeled with relative ease. The behavior of other metals of interest could be predicted based on relatively few laboratory experiments, or on approaches that enable prediction of stability constants for metals onto bacterial surfaces (Fein et al., 2001). Many of the relevant metal-binding constants and site densities for other important binding ligands such as mineral surfaces (Koretsky et al., 1998; Sahali and Sverjensky; 1998) and humic and fulvic acids (Milne et al., 2001; Milne et al., 2003) already exist.

Our results suggest that reasonable estimates of the extent of bacterial adsorption in natural environments can be obtained simply by determining the absolute concentration of all bacteria in the system, rather than by having to characterize the adsorption behavior, the distribution, and the concentration of each bacterial species present. Our results also suggest that estimations of the extent of metal adsorption based on pure cultures of individual bacterial species may be higher than the adsorption that occurs onto natural bacterial consortia. The repeated regeneration (over months or years) of single strains of bacteria using a high-nutrient growth medium may influence the density of functional group sites on their surfaces. In contrast, our approach of measuring adsorption onto consortia that have been grown under laboratory conditions for only a few generations yields thermodynamic parameters which are likely to better reflect the adsorption behavior of bacterial consortia under natural conditions. (Sahali and Sverjensky, 1998)

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