

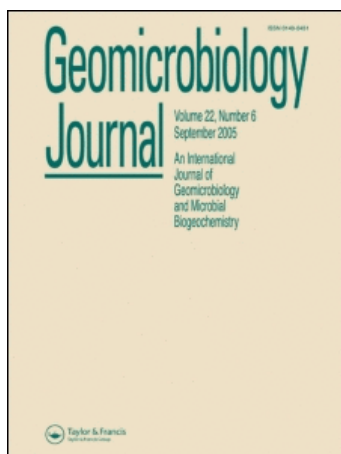
This article was downloaded by: [University of Notre Dame]

On: 5 August 2010

Access details: Access Details: [subscription number 917394054]

Publisher Taylor & Francis

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



## Geomicrobiology Journal

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713722957>

### Calibration of a Linear Free Energy Estimation Approach for Estimating Stability Constants for Metal-Bacterial Surface Complexes

Brian R. Ginn<sup>a</sup>; Jennifer E. S. Szymanowski<sup>a</sup>; Jeremy B. Fein<sup>a</sup>

<sup>a</sup> Department of Civil Engineering and Geological Science, University of Notre Dame, Notre Dame, IN, USA

Online publication date: 26 May 2010

**To cite this Article** Ginn, Brian R. , Szymanowski, Jennifer E. S. and Fein, Jeremy B.(2010) 'Calibration of a Linear Free Energy Estimation Approach for Estimating Stability Constants for Metal-Bacterial Surface Complexes', Geomicrobiology Journal, 27: 4, 321 – 328

**To link to this Article:** DOI: 10.1080/01490451003705167

**URL:** <http://dx.doi.org/10.1080/01490451003705167>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

# Calibration of a Linear Free Energy Estimation Approach for Estimating Stability Constants for Metal-Bacterial Surface Complexes

Brian R. Ginn, Jennifer E.S. Szymanowski, and Jeremy B. Fein

Department of Civil Engineering and Geological Science, University of Notre Dame, Notre Dame, IN, USA

In this study, we use the measured extent of metal adsorption onto bacterial cells to constrain a linear free energy relationship that allows estimation of unknown stability constants for metal-bacterial surface complexes based on the value of corresponding aqueous metal-acetate stability constants. A previous study (Fein et al., 2001) used metal adsorption experiments to constrain a similar relationship, but the experiments were conducted using acid-washed bacteria, and subsequent evidence (Borrok et al., 2004a) shows that the acid-washing step affects the extent of adsorption of a number of metals onto bacterial surfaces. We measured the adsorption of Zn, Ni, Co, Sr, and Nd onto *Bacillus subtilis* in 0.1 M NaClO<sub>4</sub> as a function of pH and metal:bacterial site ratio, using a non-electrostatic discrete four-site model of the bacterial protonation reactions as a basis for the metal adsorption modeling. The adsorption of the divalent cations (Zn, Ni, Co, and Sr) could best be modeled by considering adsorption reactions involving three sites on the bacterial surface; we used a one-site model to account for the Nd data that covered a more restricted pH range. The calculated stability constants for metal-Site 2 bacterial surface complexes are used to re-calibrate the linear free energy relationship previously defined by Fein et al. (2001). There is a significant difference between the original and the re-calibrated lines for weakly binding cations such as Sr<sup>2+</sup>, but the difference becomes negligible for the stronger-binding cations. Because the linear free energy relationship defined in this study was calibrated from experiments that involved bacteria that were not exposed to acidic conditions, the estimated stability constant values that result from using this relationship are likely to reasonably reflect bacterial adsorption behaviors that occur in realistic geologic settings.

**Keywords** adsorption, bacteria, free energy line, surface complexation, thermodynamic

Received 12 October 2007; accepted 13 January 2009.

Research funding was provided by the National Science Foundation through an Environmental Molecular Science Institute grant (EAR02-21966). Two journal reviews significantly improved the presentation of the research.

Address correspondence to Brian R. Ginn, Department of Civil Engineering and Geological Science, 156 Fitzpatrick Hall, University of Notre Dame, Notre Dame, IN 46556, USA. E-mail: bginn@nd.edu

## INTRODUCTION

Bacterial cell walls contain acidic functional groups that can bind and affect the mobility of aqueous cations in near-surface environments (Beveridge and Murray 1976; Fortin and Beveridge 1997; Ledin et al. 1999; Warren and Ferris 1998; Ohnuki et al. 2005). Metal speciation models can aid in understanding the impact of metal adsorption onto bacterial cell walls in a particular geochemical system, and surface complexation models (SCMs) have been developed in recent years to meet this need (e.g., Xue et al. 1988; Plette et al. 1996; Fein et al. 1997; Daughney and Fein 1998; Cox et al. 1999; Fowle and Fein 1999; Haas et al. 2001). The SCM approach requires a detailed knowledge of the number of types of functional groups responsible for metal binding, the concentrations of those sites within the cell wall, and the acidity constants of those sites. The main benefit of the SCM approach is its ability to predict the extent of adsorption under conditions not directly studied in the laboratory (Bethke and Brady 2000; Koretsky 2000).

If SCMs are mechanistically accurate, then the equilibrium constants that correspond with those adsorption reactions are invariant with respect to pH, solute-sorbent ratios, fluid composition, and ionic strength. However, the adsorptive properties of the cell wall, and hence the stability constant values for a given bacterial species can vary as a function of the stage at which the bacteria are harvested as well as the composition of the growth medium (Daughney et al. 2001; Borrok et al. 2004a). Also, the results obtained for one species may not necessarily be applicable to other species. (Ginn and Fein 2008)

SCMs are difficult to apply to natural settings due to the wide range of solutes and sorbents present in these systems and the fact that thermodynamic stability constants must be obtained for each important surface complex. To address the lack of thermodynamic data, linear free-energy relationships have been used to estimate unknown stability constant values for aqueous metal-anion complexes (e.g., Langmuir 1979; Stumm and Morgan 1996). For example, Fein et al. (2001) measured the equilibrium constants for the adsorption of Zn, Co, Ni, Sr, and Nd onto *B. subtilis*., and defined a relationship between the stability constants of the metal-bacterial surface complexes and

the stability constants of corresponding metal-acetate aqueous complexes. The results from Fein et al. (2001) suggest that unknown stability constants for a metal-bacterial surface complex can be estimated through this relationship if the metal-acetate stability constant is known for the metal of interest.

The relationship defined by Fein et al. (2001) is based on metal adsorption experiments conducted using bacteria that were washed in a dilute acid to remove competing cations from the growth medium. However, exposing bacteria to acidic conditions can affect their metal binding capabilities. Wong et al. (1993) and Chang et al. (1997) exposed two gram-negative species, *Pseudomonas aeruginosa* and *Pseudomonas putida*, to solutions with pH values as low as 2.0. They found that the bacterial surfaces exposed to acid adsorbed metals (Cd, Cu, and Pb) to a higher extent than did the bacterial surfaces that had not been exposed to acidic conditions.

Similarly, Borrok et al. (2004a), testing two gram-positive species (*B. subtilis* and *B. cereus*) and two gram-negative species (*P. aeruginosa* and *P. mendocina*), also observed an increase in metal binding capacity for bacteria rinsed in acid relative to non-acid washed bacteria. Borrok et al. (2004a) observed enhanced leaching of Ca and Mg from the bacteria under acidic conditions. They speculated that the enhanced metal adsorption was due to displacement of structurally bound Ca and Mg by protons in the acid-washed samples, thereby creating additional binding sites for aqueous metal cations. Because bacteria in most natural systems have not been exposed to acidic conditions, adsorption experiments involving bacteria that have not been acid-washed are more likely to mimic natural environments.

In this study, we use metal adsorption experiments involving non-acid washed bacteria to recalibrate the stability constant estimation relationships defined by Fein et al. (2001). In addition, we model the adsorption data using a non-electrostatic discrete-site SCM, based on bacterial site acidity constants and site concentrations from Fein et al. (2005) to obtain stability constants for the important metal-bacterial surface complexes. We compare our constants with those obtained by Fein et al. (2001), and determine the effect of the acid wash treatment on metal binding by the bacterial surface. We use the calculated stability constants to constrain a new relationship between stability constants for metal-bacterial surface complexes and those for corresponding metal-acetate aqueous complexes. The stability constant relationship in this study should better reflect metal binding behaviors in natural systems compared to the relationship defined by Fein et al. (2001).

## METHODS

### Bacterial Growth and Preparation

The bacterial species used in this study was the gram-positive species *Bacillus subtilis*, which is a common and well-studied soil species. A wide range of bacteria exhibit similar proton and metal adsorption behaviors (Yee and Fein 2001; Borrok et al. 2004a, 2004b; Johnson et al. 2007), and *B. subtilis* is a

good representative species of those species that exhibit these commonalities. The bacteria were incubated at 32°C for 24 h in 3 mL of commercially-supplied trypticase soy broth with 0.5% yeast extract. The bacteria were then transferred to 1 L of the same growth medium, and incubated for another 24 h at 32°C. The bacteria were then centrifuged at 5800 g for 10 min. The supernatant was poured out and the bacteria were rinsed 5 times in 0.1 M NaClO<sub>4</sub> (the electrolyte used in our experiment), centrifuging at 6300 g for 5 min between rinses. After the wash procedure, the bacteria were pelleted by centrifugation for 30 min at 6300 g twice to remove excess water.

### Metal Adsorption Experiments

The ionic strength of all of the experiments was buffered by performing the experiments in a 0.1 M NaClO<sub>4</sub> electrolyte solution. Batch adsorption experiments were performed as a function of pH using aqueous Ni, Co, Zn, and Sr, with fixed metal:bacteria concentration ratios. Experiments were performed for each metal at two different metal:bacteria ratios in order to place more rigorous constraints on the calculated stability constants for the important metal-bacterial surface complexes. Nd adsorption experiments were performed as a function of Nd concentration at a fixed pH and at a fixed concentration of bacteria because Nd adsorbs strongly to the bacterial surfaces, even at low pH. The Nd experiments were also performed twice, but under different pH conditions.

The metal solutions were prepared by diluting commercially purchased 1000 ppm aqueous metal standards with 0.1 M NaClO<sub>4</sub>. The pH values of the solutions were neutralized with NaOH prior to the addition of the bacteria. The bacteria were suspended in the metal solutions, and the suspensions were divided into multiple portions, each in a high density polypropylene test tube. For experiments conducted as a function of pH, the pH of each solution was adjusted by adding small aliquots of either HNO<sub>3</sub> or NaOH. The final pH range for each experiment was between approximately 2.5 and 8. pH measurements were performed using a glass electrode calibrated with NIST standard buffers (pH = 4.01, 7.00, and 10.01).

Although the ionic strength of the pH standards did not match exactly the ionic strengths of the experimental solutions, the error introduced by this discrepancy is likely minimal relative to other experimental uncertainties. For the experiments conducted as a function of Nd concentration, variable amounts of a pH neutralized parent Nd solution were added to test tubes that contained a bacterial suspension in 0.1 M NaClO<sub>4</sub> in order to yield the desired final Nd concentration range; the pH of these systems was adjusted to either approximately 5.6 or 6.7 using NaOH.

Each experimental system was allowed to equilibrate for 2 h, and the final pH was measured. Fowle and Fein (2000) demonstrated that metal adsorption reactions involving *B. subtilis* reach a reversible equilibrium in less than 1 h. After the 2 h reaction period, the test tubes were centrifuged for 2 min at 6300 g, and

filtered through a 0.45  $\mu\text{m}$  pore-size cellulose nitrate membrane. The filtered solutions were acidified with minute amounts of concentrated  $\text{HNO}_3$ , and analyzed for remaining metal content with inductively coupled plasma optical emission spectroscopy (ICP-OES). The wavelengths used to quantify the concentration of each metal were: 228.616 nm for Co, 221.647 nm for Ni, 232.238 nm for Sr, 213.857 nm for Zn, and 401.225 nm for Nd. Aqueous metal standard solutions were created using 0.1 M  $\text{NaClO}_4$  as a background electrolyte in order to avoid matrix effects on the analyses. The analytical uncertainties for the ICP-OES analyses were  $\pm 3\%$  as determined by repeat analyses of the standards for each metal during the analysis of the experimental samples. The extent of adsorption for each metal is the difference between the known starting metal concentration and the measured final metal concentration.

## RESULTS

The Sr, Co, Ni, and Zn adsorption experiments exhibit typical cation adsorption behavior as a function of pH, with adsorption increasing with increasing pH (Figure 1). The Ni adsorption experiments exhibit a reversal of this trend under the highest pH conditions studied, with adsorption decreasing with increasing pH above approximately pH 7.5 (Figure 1g). In the Nd experiments (Figure 2), the extent of Nd adsorption increases with increasing total Nd concentration in the system, with an approximately linear relationship between the log molality of adsorbed Nd and the log molality of total Nd in each experiment. For each of the metals studied, increasing the bacterial:metal concentration ratio increases the extent of adsorption, with the effect ranging from an enhancement of approximately 10% to over 25% under the conditions studied.

## DISCUSSION

### Thermodynamic Modeling

We use the adsorption data to calculate the stability constants for the important metal-bacterial surface complexes. We conduct these calculations using the program FITEQL 2.0 (Westall 1982) to account for both bacterial surface complexation and aqueous reactions in the experimental systems. These calculations account for aqueous metal hydrolysis reactions using equilibrium constants from Baes and Mesmer (1976). Activity coefficients for aqueous ions are calculated by FITEQL using the Davies equation (Westall 1982), and the program calculates a variance parameter,  $V(Y)$ , that constrains the goodness-of-fit between a model and the experimental data. In general, a  $V(Y)$  value of less than 20 indicates a reasonable model fit (Westall 1982), and we use the  $V(Y)$  values to determine the best-fitting model for each data set. We use the non-electrostatic 4-site surface protonation model proposed by Fein et al. (2005) for *B. subtilis* to account for the protonation state of the cell wall functional groups. In this model, functional group speciation is described

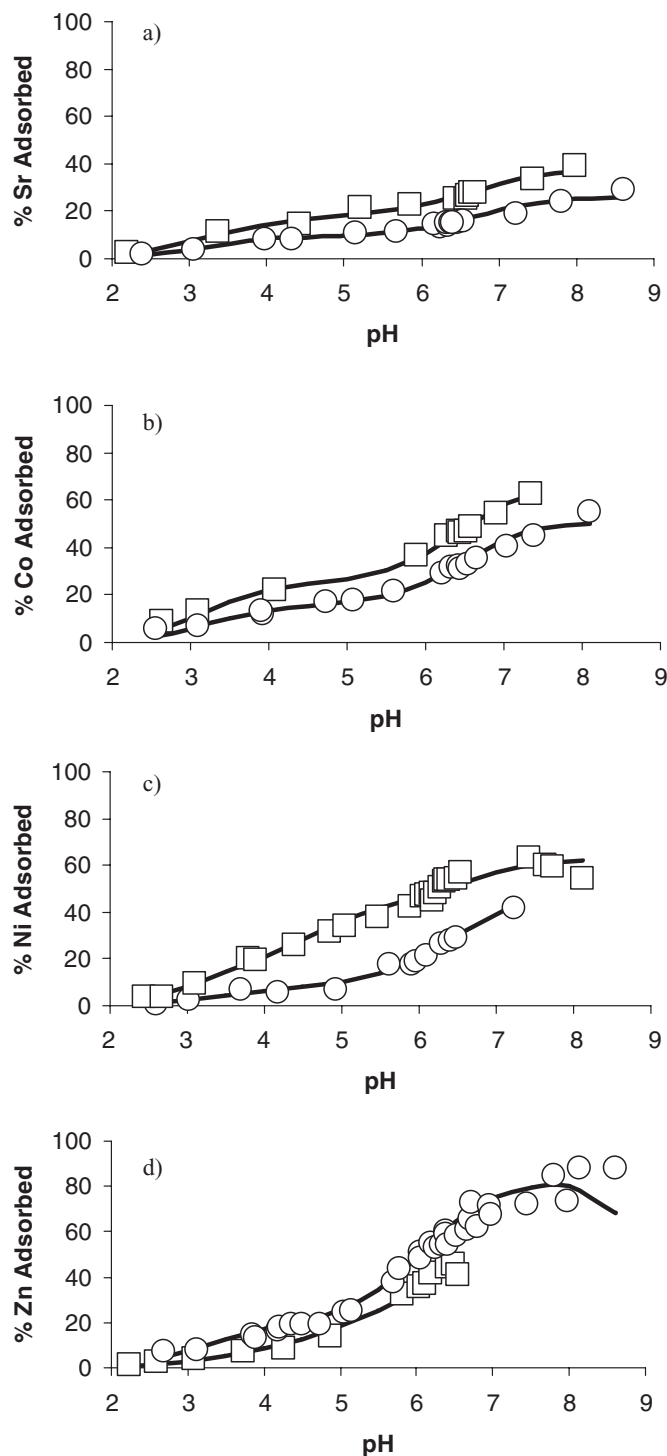


FIG. 1. Metal adsorption onto *B. subtilis*. Metals are from top to bottom: Sr, Co, Ni, and Zn. Solid curves are best fitting models of the experimental data. The background electrolyte in each experiment is 0.1 M  $\text{NaClO}_4$ . The experimental conditions are: a) 3.0 ppm Sr and 10.1 g/L bacteria (circles) and 3.0 ppm Sr and 20.1 g/L bacteria (squares); b) 10.2 ppm Co and 10 g/L bacteria (circles) and 10.2 ppm Co and 14.8 g/L bacteria (squares); c) 8.6 ppm Ni and 4.9 g/L bacteria (circles) and 8.5 ppm Ni and 15 g/L bacteria (squares); and d) 6.7 ppm Zn and 5.0 g/L bacteria (circles) and 6.6 ppm Zn and 10.0 g/L bacteria (squares).

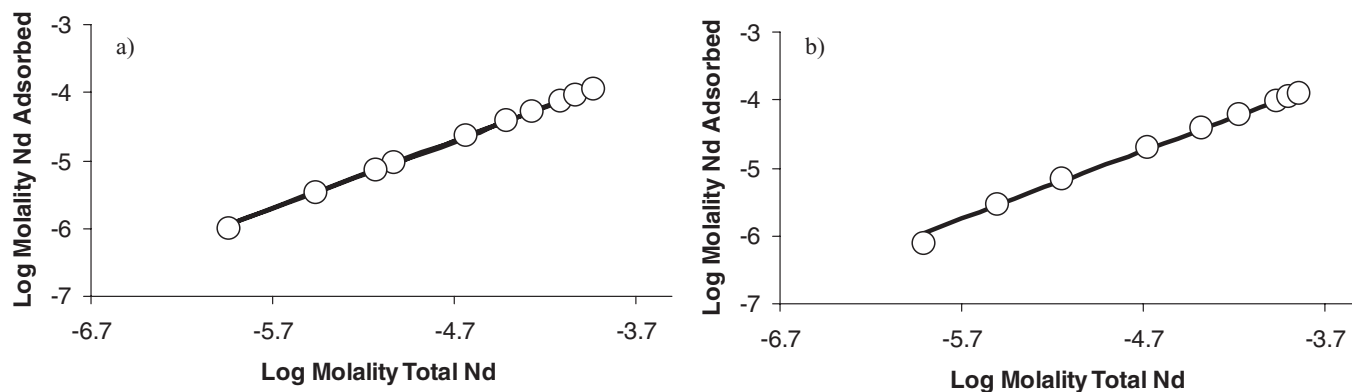
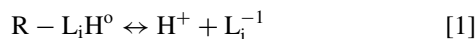


FIG. 2. Nd adsorption onto *B. subtilis*. a) pH 5.6 experiments; b) pH 6.7 experiments. The solid curves are best fitting models of the experimental data (circles). The concentration of bacteria in each experiment is 8.7 g/L wet mass and the background electrolyte is 0.1 M NaClO<sub>4</sub>.

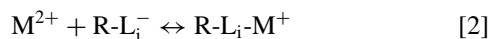
by the following reaction stoichiometry:



where  $L_i$  represents one of the four discrete site types active on the cell wall, and R represents the cell wall macromolecule to which each functional group is attached.

The equilibrium constant for reaction (1) is the acidity constant for the site type in question. The pKa (negative logarithm of the acidity constant) values for the four sites on *B. subtilis* are 3.3, 4.8, 6.8, and 9.1, respectively, and the corresponding site concentrations are  $8.1 \times 10^{-5}$ ,  $1.1 \times 10^{-4}$ ,  $4.4 \times 10^{-5}$ , and  $7.4 \times 10^{-5}$  moles of sites/gm wet mass bacteria, respectively (Fein et al. 2005), and these values form the basis for modeling the metal adsorption data. We refer to these sites as Sites 1-4, respectively. We use a non-electrostatic modeling approach to quantify adsorption onto the bacterial surface sites (e.g., Fein et al. 2005), and as such the stability constants that we calculate for the metal-bacterial surface site complexes represent conditional stability constants applicable only to the ionic strength of the experiments. Ionic strength does not exert a large effect on proton binding onto bacterial sites, but it can significantly affect the extent of binding of some metals (e.g., Borrok and Fein 2005; Fein et al. 2005).

The best-fitting models for the adsorption of Sr, Co, Ni, and Zn involve the simultaneous adsorption of the divalent metal cation of interest onto the negatively charged deprotonated form of Sites 1, 2, and 3 on the bacterial surface, according to the following reaction stoichiometry:



Models that include metal binding to fewer than these three sites, and models that include metal binding to other combinations of three sites, yield significantly higher  $V(Y)$  values, and markedly misfit the trends of the data as a function of pH. Due to the more restricted pH range of the Nd experiments, the best fitting model for the Nd isotherms involve adsorption of the metal onto the

deprotonated form of Site 2 only. Equation 2 explicitly assumes a 1:1 metal to site stoichiometry, which is consistent with findings from previous studies (e.g. Fein et al. 1997, 2001; Borrok et al. 2004a). The Nd experiments were conducted as a function of Nd concentration, and the data in theory could be used to test the reaction stoichiometry. However, because nearly 100% of the Nd in each experiment was adsorbed onto the bacterial surface, the slope of the isotherm becomes insensitive to the stoichiometry of the reaction and depends solely on the mass balance of Nd<sup>3+</sup> cations. For consistency, we model the Nd data using the same 1:1 adsorption reaction stoichiometry that we invoke to model the data for the other metals.

The solid curves in Figure 1 depict the best-fitting models for each experiment, and Table 1 compiles the calculated log K values and associated  $V(Y)$  values for each model fit. Adsorption experiments involving each metal were conducted at two different metal:bacteria concentration ratios, and Table 1 lists the log K values for each experiment for each metal, as well as the average values for each log K. The  $V(Y)$  values for all of the experiments are less than 8, and these values and the visual fits to the data both indicate that the best-fitting models provide excellent fits to the data for each metal studied here both as a function of pH and as a function of metal:bacteria ratio. For each metal, the stability constants do not change beyond the margin of error when the metal to bacteria ratio is changed, indicating that the assumed 1:1 adsorption reaction stoichiometry is consistent with the experimental data.

The uncertainties for each K value were determined by finding the lowest and highest K values that are consistent with the adsorption data. The uncertainty for each K value was determined by testing the ability of a range of K values to fit only the portion of the adsorption edge that corresponds to the proton-active pH range for the site of interest. The largest uncertainties are associated with the K values for Site 2. This site changes protonation state the most over the pH range of the experiments, so relatively small experimental uncertainties associated with the position of the adsorption edge lead to larger uncertainties in the K value calculated for this site.

TABLE 1  
Metal stability constants<sup>1</sup>

Metal	High Metal:Bacteria Ratio <sup>2</sup>				Low Metal:Bacteria Ratio <sup>3</sup>				Average		
	V(Y)	Site 1	Site 2	Site 3	V(Y)	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
Co	6.2	2.8 ± 0.2	1.8 ± 0.1	3.8 ± 0.1	3.4	2.9 ± 0.2	1.4 ± 0.2	3.9 ± 0.1	2.9 ± 0.3	1.6 ± 0.3	3.8 ± 0.1
Ni	3.6	2.7 ± 0.2	2.2 ± 0.4	4.2 ± 0.2	5.9	2.9 ± 0.2	3.1 ± 0.5	3.8 ± 0.3	2.8 ± 0.1	2.6 ± 0.5	4.0 ± 0.2
Zn	8.0	2.8 ± 0.2	2.2 ± 0.4	4.5 ± 0.1	3.8	2.7 ± 0.2	3.0 ± 0.4	4.4 ± 0.1	2.8 ± 0.1	2.6 ± 0.4	4.4 ± 0.1
Sr	0.2	2.5 ± 0.2	1.1 ± 0.2	3.2 ± 0.2	0.3	2.5 ± 0.2	1.7 ± 0.3	3.0 ± 0.1	2.5 ± 0.1	1.4 ± 0.4	3.1 ± 0.2
Nd	0.3	N/A	5.6 ± 0.3	N/A	0.7	N/A	4.9 ± 0.4	N/A	N/A	5.3 ± 0.4	N/A

1. Values given refer to log K values for Reaction (2).

2. Depicted as circles in Figure 1.

3. Depicted as squares in Figure 1.

### Recalibrated Linear Free Energy Relationship

Fein et al. (2001) used a linear free-energy approach to define a relationship between measured stability constants for metal-Site 2 bacterial surface complexes and equivalent aqueous metal-acetate stability constants. This relationship enables the estimation of unknown metal-Site 2 bacterial surface site stability constants from known metal-acetate stability constants. Acetate is a good proxy for site 2 because x-ray absorption spectroscopy studies of metal binding onto this site indicate that carboxyl binding predominates (Kelly et al. 2002; Boyanov et al. 2003). The stability constant relationship established by Fein et al. (2001) had a linear correlation coefficient of 0.97, corresponding to the following equation:

$$Y = 1.31 * X + 1.37 \quad [3]$$

where Y and X represent the logarithm of the stability constant values for the metal-Site 2 bacterial surface complexes and the metal-acetate aqueous complexes, respectively. Site 2 was the only one for which such a relationship could be defined by Fein et al. (2001) due to restrictions in the pH ranges of their experiments.

One of the primary objectives of this study was to re-calibrate the correlation between the stability constants for metal-acetate aqueous complexes and metal-bacterial surface complexes. We use the averaged calculated Ni, Zn, Sr, and Nd K values from Table 1 for an initial re-calibration of this relationship for Site 2. Similar relationships could not adequately be defined for the other bacterial surface sites due to insufficient data for stability constants other than for those of metal-Site 2 bacterial surface complexes. The stability constants for each metal-acetate complex are from Shock and Koretsky (1993).

The re-calibrated plot exhibits a strong linear relationship, with a linear correlation coefficient of 0.99 and a standard deviation of 0.27, corresponding to the equation:

$$Y = 2.39 * X - 1.06 \quad [4]$$

where, as above, Y and X represent the logarithm of the stability constant values for the metal-Site 2 bacterial surface complexes and the metal-acetate aqueous complexes, respectively. In order to obtain this relationship, the Co stability constant was neglected. The Co stability constant deviates by 2.4 standard deviations from the best fit line, and deviates substantially from the value of 3.1 reported in Borrok et al. (2004a), although the cause of this discrepancy is unknown. If we include an averaged Co stability constant with the other four from this study, the linear relationship has a correction coefficient of 0.94, and a standard deviation of 0.45, and becomes:

$$Y = 2.49 * X - 1.40 \quad [5]$$

Figure 3 shows this initial recalibrated line (Equation 5), as well as the original line obtained by Fein et al. (2001) (Equation 3). There is a significant difference between the original and the re-calibrated lines for weakly binding cations such as Sr<sup>2+</sup>, but the difference becomes negligible for the stronger-binding cations, such as Nd<sup>3+</sup>. This result is consistent with the measurements of Borrok et al. (2004a), who document significantly enhanced adsorption of Cd and Co onto acid-washed bacteria relative to that onto non-acid washed bacteria, but no effect of acid-washing on the adsorption of Pb. Fein et al. (2001) reported a log K value of 2.6 for Sr<sup>2+</sup> from experiments that used acid-washed bacteria, a value which is over 1 log unit greater than the average value listed in Table 1 from this study. In contrast, Fein et al. (2001) reported a value of 5.1 for Nd<sup>3+</sup> from experiments that involved acid-washed bacteria, which is within experimental error of the average value listed in Table 1 from this study.

Also included in Figure 3 are 5 metals whose stability constants were determined in previous studies. They are: a Cd-Site 2 log stability constant of 3.4 reported in Borrok et al. (2004a), a (UO<sub>2</sub>)<sup>2+</sup>-Site 2 log stability constant of 6.0 reported in Gorman-Lewis et al. (2005), an Al<sup>3+</sup>-Site 2 log stability constant of 5.0 reported in Fein et al. (1997), a Pb-Site 2 log stability constant

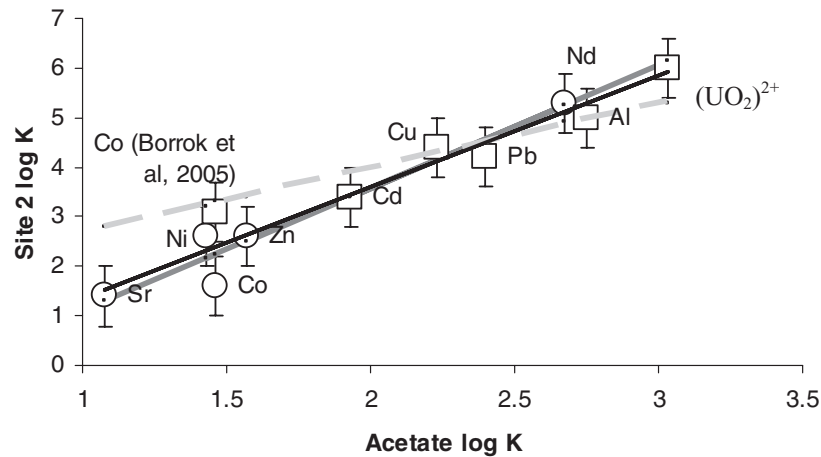


FIG. 3. Correlation plots showing metal-bacteria stability constants for *B. subtilis* versus aqueous metal-acetate stability constants. The dashed grey line represents the correlation that is derived from experiments involving acid-washed bacteria (Fein et al. 2001); the dark grey line represents the correlation based on the Ni, Zn, Sr, and Nd stability constants from this study; the black line represents the correlation based on the stability constants for Ni, Zn, Sr, and Nd from this study; Al, Pb, Cu from Fein et al. (1997); Cd from Borrok et al. (2004a); and  $(\text{UO}_2)^{2+}$  from Gorman-Lewis et al. (2005).

of 4.2 reported in Fein et al. (1997), and a Cu-Site 2 log stability constant of 4.4 reported in Fein et al. (1997). The  $\text{Cd}^{2+}$  and  $(\text{UO}_2)^{2+}$  stability constants were obtained from experiments in which the bacteria had not been acid washed. The Al, Cu, and Pb stability constants from Fein et al. (1997) were obtained from experiments in which the bacteria had been acid washed, but the K values fall in the region where little effect of acid-washing is expected. If these 5 additional cations are included, then a final linear calibration fit can be constructed with a correction coefficient of 0.98, a standard deviation of 0.24, and a corresponding equation:

$$Y = 2.23 * X - 0.84 \quad [6]$$

This best fitting line is shown in Figure 3 and is virtually indistinguishable from the best fitting line described by Equation 5, indicating that both provide excellent approximations for Site 2 stability constants.

Borrok et al. (2004a) proposed that washing bacterial surfaces under acidic conditions increases the adsorption capacity of the cell walls by irreversibly freeing  $\text{Ca}^{2+}$  and/or  $\text{Mg}^{2+}$  cations that are structurally bound within the cell wall, thereby creating additional adsorption sites. Under low pH conditions, there are enough protons in solution to make exchange between the structurally bound cations and protons energetically favorable. We propose that strongly binding metal cations such as  $\text{Nd}^{3+}$  and  $\text{UO}_2^{2+}$ , like protons under low pH conditions, can also displace structurally bound  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  from the cell wall. There is no acid-wash effect for strongly adsorbing metal cations because they are able to displace  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  and occupy their structurally-bound sites, even if the surface has not been acid washed. Conversely, weakly-adsorbing metals are not able to displace the structurally bound  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$ , and can only occupy those sites if the surface is acid

washed prior to exposure, thereby resulting in a large acid wash effect.

According to this hypothesis, the displacement of structurally-bound cations from the cell wall changes the site concentrations, but does not change the stability constant for each site type. Also, the hypothesis assumes that the stability constant is unchanged because once the structurally bound cations are irreversibly displaced, subsequent binding to the site appears to be completely reversible (Borrok et al. 2004a). That is, the calculated stability constants reflect the reversible adsorption onto these sites, and do not reflect the exchange with the structurally bound cations.

### Implications for SCMs

The effects of protons or strongly-binding cations on the adsorption behavior of bacteria present some difficulties in applying SCMs to realistic systems. The SCM approach uses estimates of site concentrations and relevant stability constants to calculate the importance of metal-surface complexes. However, our results suggest that the concentrations of sites on bacterial cell walls are enhanced in response to exposure of the cell wall to high concentrations of protons (acidic conditions) or to high concentrations of strongly-sorbing cations. In effect, different cations would experience different concentrations of the same type of site on the cell wall depending on the strength of the chemical bond formed between the cation and the sites.

It is unclear how to account for variable effective site concentrations such as this in a model of a realistic system. The accuracy of stability constant values themselves is difficult to judge. For example, in this study, we based stability constant calculations on surface site concentrations that were determined from potentiometric titration curves. However, it is difficult to determine the number of structurally-bound cation sites that were

displaced by protons during those titration experiments, and it is impossible to know if more might have been displaced if the potentiometric titration were conducted to lower pH conditions. In fact, displacement of these structurally bound cations may be the dominant proton-activity under low pH conditions. The site concentrations that we assume in this study in order to calculate metal-bacterial surface stability constants exert a significant effect on the calculated stability constant values. Therefore, the stability constant values reported here do not necessarily represent true equilibrium constants, but rather they are conditional constants derived from a model in which a fixed concentration of surface sites was assumed.

Despite the difficulties in applying SCMs to realistic settings, the models are still useful for understanding bacterial adsorption and for quantifying the effects of bacterial adsorption on metal speciation in geologic systems. The uncertainties associated with the site concentrations and stability constant values are significant, but they are likely not the largest uncertainties associated with the application of SCMs to realistic settings. As Borrok et al. (2004a) demonstrate, ignoring factors such as bacterial speciation, ionic strength, temperature, and growth conditions introduces relatively small uncertainties when modeling metal-bacterial adsorption compared to the unavoidable uncertainty associated with the determination of cell abundances in realistic geologic systems.

Although the effect noted here of variable site concentrations represents an additional increase to the overall uncertainty of speciation calculations, this may be an acceptably small increase considering the inherent difficulties in modeling complex realistic settings. Hence, we propose that reasonable estimates of the extent of bacterial cell wall adsorption can be made using the stability constants from this study, as they were derived using bacteria that most closely represent the site concentrations likely on bacterial cell walls in the field.

## CONCLUSIONS

This study indirectly quantifies the effect of acid-washing on the adsorption of metals onto a common bacterial surface. Our results suggest that the more strongly a metal binds to a bacterial surface, the smaller the effect that acid-washing exerts on the observed extent of adsorption. Acid-washing the bacterial surface increases its adsorption capacity for weakly binding metals, likely because the acid wash removes structurally bound  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  cations from the cell wall, making more sites available for adsorption.

However, because strongly bound metals do not exhibit an acid-wash effect, it is likely that they, like the high concentration of protons found under acidic conditions, are capable of displacing these structurally bound cations from the cell wall. The linear free energy relationship constrained by our calculated stability constants offers a means for estimating the bacterial adsorption behavior of metal cations that have not been studied in the laboratory. Because the relationship was calibrated using bacteria that were not exposed to acidic conditions prior to the

experiments, the estimated stability constants are more likely to reasonably reflect adsorption behaviors that occur in realistic geologic settings than the previous relationship.

## REFERENCES

- Baes CF, Mesmer RE. 1976. *The Hydrolysis of Cations* Wiley, New York.
- Bethke CM, Brady PV. 2000. How the Kd approach undermines ground water cleanup. *Ground Water* 38:435–443.
- Beveridge TJ, Murray RGE. 1976. Uptake and retention of metals by cell walls of *Bacillus subtilis*. *J Bacteriol* 127:1502–1518.
- Borrok DM, Fein JB. 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 Coll Interf Sci* 286:110–126.
- Borrok D, Fein JB, Tischler M, O'Loughlin E, Meyer H, Liss M, Kemner KM. 2004a. The effect of acidic solutions and growth conditions on the adsorptive properties of bacterial surfaces. *Chem Geol* 209:107–119.
- Borrok D, Fein JB, Kulpa Jr. CF. 2004b. Proton and Cd adsorption onto natural bacterial consortia: testing universal adsorption behavior. *Geochim Cosmochim Acta* 68:3231–3238.
- Boyanov MI, Kelly SD, Kemner KM, Bunker BA, Fein JB, Fowle DA. 2003. Adsorption of cadmium to *Bacillus subtilis* bacterial cell walls: a pH-dependent X-ray absorption fine structure spectroscopy study. *Geochim Cosmochim Acta* 67:3299–3311.
- Chang J, Law R, Chang C. 1997. Biosorption of lead, copper and cadmium by biomass of *Pseudomonas aeruginosa* PU21. *Water Res* 31:1651–1658.
- Cox JS, Smith DS, Warren LA, Ferris FG. 1999. Characterizing heterogeneous bacterial surface functional groups using discrete affinity spectra for proton binding. *Environ Sci Technol* 33:4514–4521.
- Daughney CJ, Fein JB. 1998. The effect of ionic strength on the adsorption of  $\text{H}^+$ ,  $\text{Cd}^{2+}$ ,  $\text{Pb}^{2+}$ , and  $\text{Cu}^{2+}$  by *Bacillus subtilis* and *Bacillus licheniformis*: A surface complexation model. *J Coll Interf Sci* 198:53–77.
- Daughney CJ, Fowle DA, Fortin D. 2001. The effect of growth phase on proton and metal adsorption by *Bacillus subtilis*. *Geochim Cosmochim Acta* 65:1025–1035.
- Fein JB, Boily J-F, Yee N, Gorman-Lewis D, Turner BF. 2005. Potentiometric titrations of *Bacillus subtilis* cells to low pH and a comparison of modeling approaches. *Geochim Cosmochim Acta* 69:1123–1132.
- Fein JB, Daughney CJ, Yee N, Davis T. 1997. A chemical equilibrium model for metal adsorption onto bacterial surfaces. *Geochim Cosmochim Acta* 61:3319–3328.
- Fein JB, Martin AM, Wightman PG. 2001. Metal adsorption onto bacterial surface: Development of a predictive approach. *Geochim Cosmochim Acta* 65:4267–4273.
- Fortin D, Beveridge TJ. 1997. Role of the bacterium *Thiobacillus* in the formation of silicates in acidic mine tailings. *Chem Geol* 141:235–250.
- Fowle DA, Fein JB. 1999. Competitive adsorption of metals onto bacterial surfaces: Application of a surface complexation approach. *Geochim Cosmochim Acta* 63:3059–3067.
- Fowle DA, Fein JB. 2000. Experimental measurements of the reversibility of metal-bacteria adsorption reactions. *Chem Geol* 168:27–36.
- Ginn BR, Fein JB. 2008. The effect of species diversity on metal adsorption onto bacteria. *Geochim Cosmochim Acta* 72:3939–3948.
- Gorman-Lewis D, Elias PE, and Fein JB. 2005. Uranium adsorption onto *Bacillus subtilis* bacterial cells. *Environ Sci Technol* 39:4906–4912.
- Haas JR, Dichristina TJ, Wade Jr. R. 2001. Thermodynamics of U (VI) sorption onto *Shewanella putrefaciens*. *Chem Geol* 180:33–54.
- Johnson KJ, Szymanowski JES, Borrok D, Huynh TQ, Fein JB. 2007. Proton and metal adsorption onto bacterial consortia: Stability constants for metal-bacterial surface complexes. *Chem Geol* 239:13–26.
- Kelly SD, Boyanov MI, Fein JB, Fowle DA, Yee N, Kemner KM. 2002. X-ray absorption fine structure determination of pH-dependent

- U-bacterial cell wall interactions. *Geochim Cosmochim Acta* 66:3855–3871.
- Koretsky C. 2000. The significance of surface complexation reactions in hydrologic systems: a geochemist's perspective. *J Hydrol* 230:127–171.
- Langmuir D. 1979. Techniques of estimating thermodynamic properties for some aqueous complexes of geochemical interest. In: Jenne EA, editor. *Chemical Modeling in Aqueous Systems*. American Chemical Society. P 353–387.
- Ledin M, Krantz-Rülcker C, Allard B. 1996. Zn, Cd, and Hg accumulation by microorganisms. Organic and inorganic soil components in multi-compartment systems. *Soil Biol Biochem* 28:791–799.
- Ohnuki T, Yoshida T, Ozaki T, Samadfam M, Kozai N, Yubuta K, Mitsugashira T, Kasama T, Francis AJ. 2005. Interactions of uranium with bacteria and kaolinite clay. *Chem Geol* 220:237–243.
- Plette ACC, van Riemsdijk WH, DeWit JCM, Benedetti MF. 1995. pH dependent charging behavior of isolated cell walls of a gram-positive soil bacterium. *Appl Geochem* 18:527–538.
- Schock EL, Koretsky CM. 1993. Metal-organic complexes in geochemical processes: Calculation of standard partial molal thermodynamic properties of aqueous acetate complexes at high pressures and temperatures. *Geochim Cosmochim Acta* 57:4899–4922.
- Stumm W, Morgan JJ. 1996. *Aquatic Chemistry*, 3rd ed. New York: Wiley.
- Warren LA, Ferris FG. 1998. Continuum between sorption and precipitation of Fe(III) on microbial surfaces. *Environ Sci Tech* 32:2331–2337.
- Westall JC. 1982. *FITEQL, a Computer Program for Determination of Chemical Equilibrium Constants from Experimental Data*. Version 2.0. Report 82-02. Department of Chemistry, Oregon State University.
- Wong PK, Lam KC, So CM. 1993. Removal and recovery of Cu(II) from industrial effluent by immobilized cells of *Pseudomonas putida* II-11. *Appl Microbiol Biotechnol* 39:127–131.
- Xue H-B, Stumm W, Sigg L. 1988. The binding of heavy metals to algal surfaces. *Water Res* 22:917–926.
- Yee N, Fein JB. 2001. Cd adsorption onto bacterial surfaces: A universal adsorption edge? *Geochim Cosmochim Acta* 65:2037–2042.