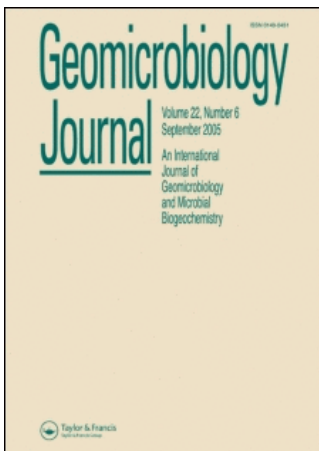


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Geomicrobiology Journal

Publication details, including instructions for authors and subscription information:
<http://www.informaworld.com/smpp/title~content=t713722957>

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Online Publication Date: 01 December 2004

To cite this Article: Ams, David, Fein, Jeremy, Dong, Hailiang and Maurice, Patricia (2004) 'Experimental Measurements of the Adsorption of *Bacillus subtilis* and *Pseudomonas mendocina* Onto Fe-Oxyhydroxide-Coated and Uncoated

Quartz Grains', *Geomicrobiology Journal*, 21:8, 511 — 519

To link to this article: DOI: 10.1080/01490450490888172

URL: <http://dx.doi.org/10.1080/01490450490888172>

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Experimental Measurements of the Adsorption of *Bacillus subtilis* and *Pseudomonas mendocina* Onto Fe-Oxyhydroxide-Coated and Uncoated Quartz Grains

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In this study, we compared the adsorption of the gram-positive bacterium *Bacillus subtilis* with adsorption of the gram-negative bacterium *Pseudomonas mendocina* onto Fe-oxyhydroxide-coated and uncoated quartz grains as a function of pH and bacteria : mineral mass ratio. We studied metabolically-inactive cells in order to focus on the initial bacterial attachment mechanisms. The data show that the presence of Fe-oxyhydroxide-coatings on quartz surfaces significantly enhances the adsorption of bacteria and that in general the extent of adsorption decreases with increasing pH and with decreasing bacteria : mineral mass ratio. *B. subtilis* adsorbs to a greater extent than does *P. mendocina* onto the surface of the Fe-coated quartz. The adsorption behavior appears to be controlled by the overall surface charge of both bacterial and mineral surfaces. We model the adsorption data using a semi-empirical chemical equilibrium model that accounts for the site speciation of the adsorbing surfaces. Models describing bacterial adsorption to Fe-oxyhydroxide-coated quartz can account for changes in pH and bacteria : mineral mass ratio using one set of equilibrium constants.

Keywords bacteria, adsorption, *Bacillus subtilis*, *Pseudomonas mendocina*, Fe-oxyhydroxide-coatings, quartz, surface complexation model

Received 7 May 2004; accepted 28 June 2004.

Acknowledgment is made to the Donors of the Petroleum Research Fund, administered by the American Chemical Society, for partial support of this research. The Department of Energy, Division of Basic Energy Sciences, also provided partial funding. We thank Larry Hersman for providing us with the original culture of *Pseudomonas mendocina*, and for useful discussions. Two anonymous journal reviews were constructive and significantly improved the presentation of the manuscript. UV-Vis spectrophotometry measurements were conducted in the Center for Environmental Science and Technology at University of Notre Dame.

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INTRODUCTION

The extent of bacterial adsorption onto mineral surfaces can affect the mobility and ultimate fate of contaminants in geologic systems. Bacterial cell walls can adsorb high concentrations of aqueous metal cations (e.g., Beveridge and Murray 1976, 1980; Beveridge and Koval 1981; Crist et al. 1981; Harvey and Leckie 1985; Goncalves et al. 1987; Fein et al. 1997; Yee and Fein 2001) and organic contaminants (e.g., Chacko and Lockwood 1967; Johnson and Kennedy 1973; Baughman and Paris 1981; Lal and Saxena 1982; Lindqvist and Enfield 1992; Daughney and Fein 1998) under a range of pH conditions. Adsorption of contaminants to bacterial surfaces links the mobility of the contaminant to the mobility of the bacteria (e.g., Lindqvist and Enfield 1992; Yee and Fein 2002). Furthermore, bacterial attachment to mineral surfaces represents an initial step in bacterial colonization and biofilm formation processes (e.g., Forsythe et al. 1998).

The presence of Fe-oxyhydroxide-coatings on mineral surfaces is common in natural aquifer sediments (e.g., Barber et al. 1992; Ryan and Gschwend 1992; Scholl and Harvey 1992). A number of laboratory studies have shown that bacterial adsorption is dramatically enhanced in the presence of Fe-oxyhydroxide-coated quartz grains relative to pure quartz grains (e.g., Mills et al. 1994; Johnson and Logan 1996; Knapp et al. 1998). Both quartz and bacteria exhibit negatively-charged surfaces, while Fe-oxyhydroxide surfaces are dominantly positively charged under most environmentally relevant pH conditions. Therefore, it seems likely that the observed effect of mineralogy on bacterial adsorption is at least affected, if not controlled, by electrostatic forces between the interacting surfaces. Solution pH controls surface charge on both mineral and bacterial surfaces, yet only a few studies have focused on the effect of pH on bacterial adsorption to natural materials (e.g., Scholl et al. 1990; Scholl and Harvey 1992; Yee et al. 2000). Yee et al. (2000) show bacterial adsorption onto corundum to be strongly pH

dependent, with adsorption significantly decreasing with increasing pH. Scholl and Harvey (1992) show a similar bacterial adsorption trend with pH in the presence of natural Fe-, Al-, Mn-, and organic-coated glacial sand.

The effects of cell wall structure on bacterial adsorption to mineral surfaces have also yet to be fully elucidated. Because adsorption is a surface related phenomenon, the differences in the cell wall structures between gram-positive and gram-negative bacteria may be reflected in bacterial adsorption behavior. Many bacterial adsorption studies have been performed using either gram-positive or gram-negative bacteria, but very few studies have compared the adsorption behavior of the two. In the only comparative adsorption study to date involving Fe-oxyhydroxide-coated quartz, Mills et al. (1994) showed that both the gram-positive species W8 and gram-negative species S1 exhibit 100% adsorption to Fe-oxyhydroxide-coated quartz grains under the conditions of the experiments. However, Mills et al. (1994) observed greater adsorption of W8 to uncoated quartz than S1, and the difference between the adsorption behaviors of these species increased with increasing ionic strength. Only at low ionic strength (.01 M) was the extent of adsorption found to be equal for the two bacterial species. However, to date, no studies have been performed that compare the adsorption of gram-positive and gram-negative bacteria as a function of pH and mass ratio.

One of the objectives of this study is to formulate a model of bacterial adsorption behavior that can account for the effects of pH and bacteria : mineral ratios. Bulk partitioning approaches are commonly used to describe bacterial adsorption onto mineral surfaces (e.g., Walker et al. 1989; Ohmura et al. 1993; Mills et al. 1994). The partition coefficients determined in these approaches are system specific, and therefore cannot be extrapolated to predict the extent of adsorption under conditions other than those directly studied in the laboratory. Bacterial adsorption behavior has been shown to be sensitive to changes in solution ionic strength, pH, mineralogy, and bacterial concentration (e.g., van Loosdrecht et al. 1989; Scholl et al. 1990; Scholl and Harvey 1992; Mills et al. 1994; Yee et al. 2000), so flexible models are needed to account for these effects. Recently, a surface complexation approach has been used successfully to model bacterial adsorption to mineral surfaces (Yee et al. 2000; Yee and Fein 2002). In this approach, adsorption is described using a balanced adsorption reaction, and the extent of adsorption is modeled with the equilibrium constant, K , for this reaction. The advantage of surface complexation models is that a single set of K values can be used to estimate the effects of pH and bacteria : mineral mass ratio on the extent of adsorption.

Bacterial adsorption onto mineral surfaces in geologic systems results from a combination of metabolic and nonmetabolic processes. Metabolizing cells of a number of species produce a variety of exopolymers to form biofilms that aid in the attachment of bacteria under nonfavorable electrostatic conditions. For instance, Forsythe et al. (1998) observe extensive biofilm forma-

tion and a variety of attachment features associated with the adsorption of metabolizing *P. mendocina* cells to Fe-oxyhydroxide surfaces at a pH above the pH_{zpc} of the minerals. While metabolic processes may control the long-term adhesion behavior of bacteria, nonmetabolic processes such as electrostatic interactions and bacterial straining through the geologic matrix are likely to influence or control the initial attachment of bacteria onto mineral surfaces.

The objective of this study is to determine the effect of Fe-oxyhydroxide-coatings on the adsorption of nonmetabolizing bacteria to quartz as a function of pH and bacteria : mineral ratio, and to compare the adsorption behaviors of gram-positive and gram-negative bacterial species. We perform batch bacterial adsorption experiments as a function of time, pH, and bacteria : mineral mass ratio. Desorption experiments are also performed in order to ascertain the extent of reversibility of the adsorption process. Furthermore, our goal is to apply a surface complexation model to account for the observed pH effects, and thereby to provide insight into the mechanisms that control the bacterial adsorption. The use of nonmetabolizing bacteria can provide insight into the initial bacterial attachment to surfaces and can simulate the adsorption of bacteria under nutrient-depleted circumstances.

METHODS

Mineral Preparation

The quartz sand used in these experiments was obtained from Accusand. These uncoated quartz grains were washed in the same manner as described by Yee et al. (2000). Briefly, grains were washed in 1 M NaOH, rinsed 3 times with ultrapure 18 M Ω water, washed again with 10% HNO₃, then washed in ultrapure water repeatedly until the pH of the supernatant remained constant. The surface area of quartz grains was previously determined by BET isotherm analysis to be 0.2249 m²/g (Yee et al. 2000). Fe-oxyhydroxide-coated quartz grains were prepared by precipitation of a Fe phase onto the same sand grains used in the quartz experiments. Briefly, quartz grains were added to a 2 L Pyrex glass flask filled with 1 L of a .05 M Fe(NO₃)₃·9H₂O solution. While stirring, the Fe(NO₃)₃·9H₂O plus quartz mixture was titrated up pH slowly with NaOH to pH ~6 where the solubility of Fe(III) is low. The solution was then left to stir. After 24 h the solution was decanted to waste and the remaining coated quartz grains washed three times in ultrapure water. The coated quartz grains were then coated a second time in the same manner as before. After the second coating, the quartz grains were washed 20 times with ultrapure water. The grains were then left to air-dry overnight. X-ray diffraction analysis indicated the Fe-precipitate to be amorphous, and we refer to the Fe-oxyhydroxide coating hereinafter simply as an Fe-coating. X-ray diffraction data were collected using a Rigaku Miniflex diffractometer from 6 to 120° 2 θ ; with a step-width of 0.01° and 1 s spent counting per step.

Bacterial Growth and Washing Procedures

The bacterial species used in this study are *Bacillus subtilis* and *Pseudomonas mendocina*. *B. subtilis* is a gram-positive aerobic species commonly found in the subsurface. *P. mendocina* is a gram-negative aerobic species isolated from sediment in a surface holding pond from a drilling operation at the Nevada Test Site, NV, USA (Hersman et al. 2001). The bacterial growth and washing procedures that we followed have been described previously (e.g., Yee et al. 2000). Briefly, a starter culture of each bacterium in a trypticase soy broth/yeast extract medium was incubated at 32°C over 2 days to early stationary phase. Cells were then removed by centrifugation, followed by several washings in 0.1 M NaClO₄ and one acid wash (HNO₃) step at pH 1.5 to remove adsorbed cations from the cell walls. Previous studies have indicated that washed cells are intact and nonmetabolizing (e.g., Yee and Fein 2001). The wet weight of cells was measured after a final centrifugation at 7,500 rpm for 1 h. *P. mendocina* cells were prepared following the same procedure, but without the acid-wash step in order to avoid excessive disruption of the more fragile gram-negative cell wall structure. The wet weight of each type of bacterial cell is approximately 5 times more than the dry weight of the biomass (Borrok et al. 2004).

Bacteria-Mineral Adsorption Experiments

We conducted bacterial adsorption experiments onto either uncoated or Fe-coated quartz grains as a function of pH and bacteria : mineral mass ratio, following a procedure similar to that used by Yee et al. (2000). In experiments that were conducted as a function of pH, a stock suspension of either *B. subtilis* or *P. mendocina* was prepared by mixing a known weight of bacteria in enough 0.1 M NaClO₄ electrolyte solution to yield 1 g of bacteria (wet weight) per liter of solution. Then, 8 g of mineral grains were added to clean Teflon test tubes, followed by the addition of 5 mL of the 1 g/L bacterial stock solution. The pH of each reaction vessel was adjusted to a desired value (within the range pH 2–9) with small aliquots of 0.1 M NaOH and/or 0.1 M HNO₃. In the experiments conducted as a function of bacteria : mineral mass ratio, varying amounts of a concentrated bacterial stock solution were added to reaction vessels and diluted to desired concentrations of 2.0, 1.5, 1.0, 0.8, and 0.5 g bacteria/L with 0.1 M NaClO₄ to a total volume of 5 mL. The pH of samples in the bacteria : mineral mass ratio experiments were all approximately 6.0 ± 0.5, and were left unadjusted prior to equilibration. In each type of experiment, the bacteria-electrolyte-mineral mixtures were allowed to equilibrate for 2 h while being rotated and mixed periodically by hand. Preliminary experiments indicated that equilibrium time for these systems was less than 2 h.

Immediately after the 2-h equilibration period, the pH of the individual experiments was measured. Reaction vessels were then rotated end-over-end a final time to ensure that unattached bacteria were evenly distributed throughout the solution. Mineral grains settled to the bottom of the reaction vessels within

seconds of completing the end-over-end rotation, resulting in a uniform suspension of nonadsorbed bacteria in the overlying solution. A 1 mL aliquot was then removed from each reaction vessel for determination of nonadsorbed bacterial concentrations by UV-Vis spectrophotometry. UV/Vis absorption measurements were made on a Varian Cary 3 double-beam spectrophotometer, using 1.0 cm quartz cells with deionized water as the reference and for the baseline correction. Light scattering was measured at a wavelength of 400 nm and compared with measurements of 5 standards of known concentrations of suspended bacteria. Concentrations of standards were chosen to span the entire range of expected experimental values (1.0, 0.8, 0.5, 0.3, and 0.2 g/L) and were made with the same electrolyte used in the experiments. The pH value of each standard was approximately 6.5 and was not adjusted. The concentration of bacteria adsorbed onto mineral grains was determined by difference between the initial and the final nonadsorbed bacterial concentrations. Control experiments with no mineral grains present were also performed for each day's experiments in order to determine the extent to which the bacteria adsorbed onto the reaction vessel walls. This amount of bacterial loss did not vary as a function of pH over the pH range of the experiment. The average adsorption of three controls was subtracted from each experiment for each day to account for loss of bacteria.

Desorption experiments were performed for *B. subtilis* adsorption onto Fe-coated quartz in a multiple step process. An adsorption step was performed initially in the same manner as described previously. The initial adsorption step was performed at pH ~4 because our experiments showed that adsorption plateaus below pH 6–7. After a 2-h adsorption period, the pH of the experimental solution was increased to between 7 and 8 and allowed to equilibrate for another 2 h. After the 2-h desorption period, the solution concentration of bacteria was measured as described before.

Potentiometric Titrations

The amphoteric surface properties of both *B. subtilis* and *P. mendocina* have been studied and characterized previously (e.g., Borrok and Fein 2004; Fein et al. 2004, respectively). We were unable to perform titrations on the Fe-coated quartz because the large particle size of the quartz grains (required to promote settling during the adsorption experiments) made it impossible to maintain sufficient suspension or to include enough of the low surface area material in a titration vessel. Instead, an Fe-powder was titrated in order to gain information about the protonation/deprotonation behavior of a comparable Fe-mineral surface. This powder was precipitated in the same manner as in the Fe-coating procedure, but in the absence of quartz. The resulting precipitate was dried and powdered, then titrated at a concentration of 50 g mineral powder per liter in 0.1 M NaClO₄. The electrolyte solution was bubbled with N₂ gas for 1 h prior to use, and the titration cell containing the mineral suspension was kept under a N₂ atmosphere during the experiment. The sample

was continuously stirred with a small magnetic stir bar during the titration. The titrations were first run down-pH with aliquots of 0.990 N HCl to approximately pH 2.5, then up-pH with aliquots of 0.993 N NaOH to approximately pH 9.5. Each addition of acid or base occurred only after a stability of 0.1 mV/sec was attained. The BET surface area obtained for the powder was 338 m²/g, and the Fe surface site concentrations of the coated sand grains were estimated using the surface area ratio of the two Fe materials. BET surface area was measured with a model SA3100 volumetric sorption analyzer from Coulter Instruments, Inc. using N₂ adsorption at -195°C.

Zetapotential

Electrophoretic mobility measurements of *B. subtilis* and *P. mendocina* were performed with a Coulter DELSA 440 SX (Coulter Corporation, Miami, FL) in the same electrolyte (0.1 M NaClO₄) used in the adsorption experiments. The experimental conditions were as follows: temperature: 25°C, frequency range: 500 Hz, electric field strength: 6 V, on-time: 2.5 s, and off-time 0.5 s (Dong et al. 2002). The conductivity of the bacterial electrolyte solution was approximately 9.5 mS cm⁻¹. To test the pH dependence of electrophoretic mobility, pH was adjusted with HCl and NaOH prior to the mobility measurements. Reported mobility values and corresponding standard deviations represent averages of nine measurements taken from three independent samples of the same bacteria-electrolyte solution. Zetapotential values were determined from electrophoretic mobility measurements using the Helmholtz-Smoluchowski equation (Hunter 1981).

RESULTS

Bacterial Adsorption Experiments

The extent of adsorption of both *B. subtilis* and *P. mendocina* onto a fixed concentration of Fe-coated quartz is proportional to the total concentration of bacteria in the system over the concentration range studied here (Figure 1). The adsorption behavior of *P. mendocina* is similar to that observed for *B. subtilis* except that the extent of bacterial adsorption is significantly less than that observed for the same total bacterial concentration in the *B. subtilis* system.

Figures 2 and 3 show the extent of adsorption of *B. subtilis* and *P. mendocina*, respectively, onto Fe-coated and uncoated quartz as a function of pH. Both *B. subtilis* and *P. mendocina* exhibit pH dependent adsorption behavior in the presence of Fe-coated quartz. The extent of *B. subtilis* adsorption onto Fe-coated quartz is greatest between pH 4–6 where adsorption plateaus between approximately 40–65%. Above pH 6, *B. subtilis* adsorption decreases with increasing pH to pH 9, where adsorption reaches a minimum of 0–15%. The extent of adsorption of *P. mendocina* onto Fe-coated quartz is significantly less than that observed for *B. subtilis*. The extent of *P. mendocina* adsorption to Fe-coated quartz is greatest between pH 4–7.5 where adsorption plateaus

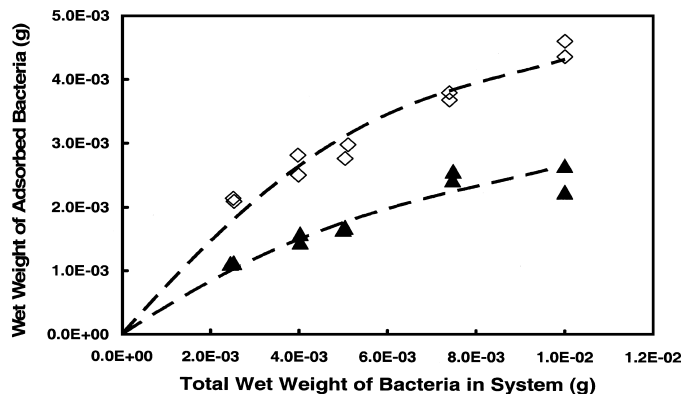


Figure 1. Bacteria:mineral mass ratio experiments. Experiments conducted with a fixed amount of Fe-coated quartz (1600 g/L) and varying amounts of bacteria 0.1 NaClO₄ electrolyte solution at pH 6.5. Open diamonds represent *B. subtilis* adsorption, closed triangles represent *P. mendocina* adsorption. Dashed curves represent model fits.

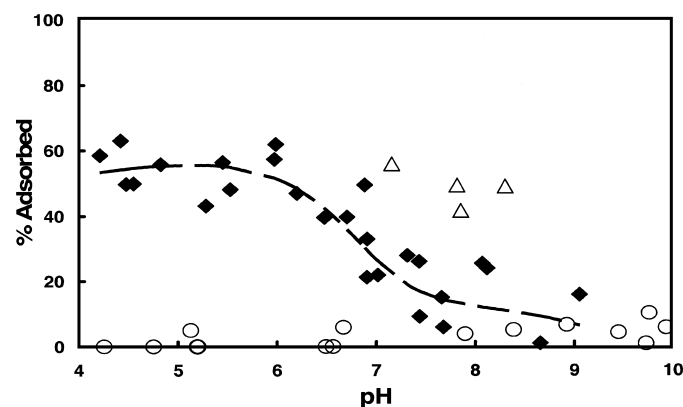


Figure 2. *B. subtilis* adsorption to Fe-coated and uncoated quartz vs. pH. Closed diamonds and open circles represent adsorption onto Fe-coated and uncoated quartz, respectively. Open triangles represent desorption measurements. Dashed curve represents the best fitting combined-site model.

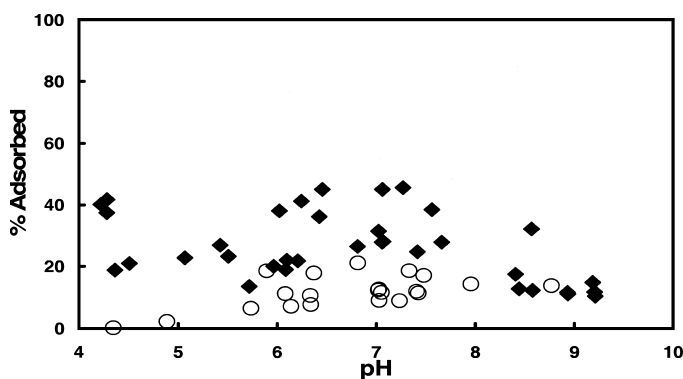


Figure 3. *P. mendocina* adsorption to Fe-coated and uncoated quartz vs. pH. Closed diamonds and open circles represent adsorption onto Fe-coated and uncoated quartz, respectively.

between 20–45%. Above pH 7.5, *P. mendocina* adsorption decreases with increasing pH to pH 9, where adsorption reaches a minimum of 10–15%. Both *B. subtilis* and *P. mendocina* exhibit a significantly lower affinity for the uncoated quartz surface. Yee et al. (2000) also observed a low affinity of *B. subtilis* for the quartz surface.

The large experimental uncertainties associated with the data shown in Figures 2 and 3 (relative, for instance, to values associated with metal adsorption experiments involving bacterial or mineral surfaces) reflect the limitations of the procedure that we use to separate adsorbed and suspended bacteria. Complete separation of suspended bacteria from adsorbed bacteria was likely limited by the inability to completely remove cells from interstitial spaces between mineral grains. However, even with these relatively high experimental uncertainties, the observed adsorption trends are significant and distinct.

The results of the desorption experiments indicate that the adsorption of *B. subtilis* onto Fe-coated quartz is irreversible over the timeframe of the experiments. That is, the extent of bacterial adsorption that was observed prior to the desorption step was virtually unchanged from that observed after adjustment to higher pH and the 2-h desorption reaction time (Figure 2). If the adsorption was even partially reversible, then the final bacterial solution concentration at the end of the desorption step would be less than that observed after the initial adsorption step. In similar experiments, Yee et al. (2000) observed complete and rapid reversibility of adsorption of *B. subtilis* onto corundum. However, other researchers have also observed irreversible adsorption behavior of bacteria onto Fe-bearing phases (e.g., Scholl and Harvey 1992; Mills et al. 1994; Johnson and Logan 1996; Knapp et al. 1998). The cause of the discrepancy between reversible adsorption in the presence of corundum and irreversible adsorption in the presence of Fe-coated quartz is unknown. While the observed pH dependence of adsorption suggests that electrostatic interactions play a significant role in bacterial attachment to Fe surfaces, the lack of reversibility suggests that the adsorption mechanism is more complex. Due to the weak pH dependence of the adsorption reaction, desorption experiments with *P. mendocina* could not be performed.

Potentiometric Titrations

Potentiometric titrations yield constraints on the site concentrations and deprotonation constants of the important functional groups on a proton-active surface. Fein et al. (2004) and Borrok and Fein (2004) modeled titration data for *B. subtilis* and *P. mendocina*, respectively, modeling the data using four discrete functional group types present on the cell wall. In this study, three titrations of suspensions of the Fe-powder were also performed in order to gain insight into the types, concentration, and deprotonation constants of sites associated with the Fe-coated quartz mineral surface. The three titration curves were virtually identical to each other, and a representative curve of all three titrations is depicted in Figure 4. Significant solution buffering over the

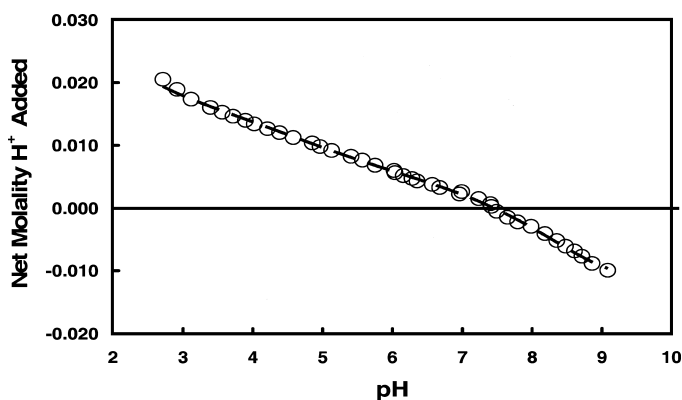


Figure 4. Potentiometric titration data from one of three titrations performed using Fe-oxyhydroxide powder. Dashed curve represents model fit.

entire pH range was observed for the Fe powder. The electrolyte used in these experiments exhibits no buffering capacity in the pH range of the study. Therefore, all observed buffering is due to the protonation and deprotonation of functional groups on the Fe-powder surface.

Zetapotential

Figure 5 shows the pH dependence of the electrophoretic mobility of *B. subtilis* and *P. mendocina*. Because electrophoretic mobility is directly related to the zetapotential of the cell, these data strongly suggest that both bacterial surfaces exhibit a negative surface charge that becomes increasingly negative with increasing pH. *P. mendocina* exhibits a significantly lower negative charge than does *B. subtilis* over the entire pH range studied.

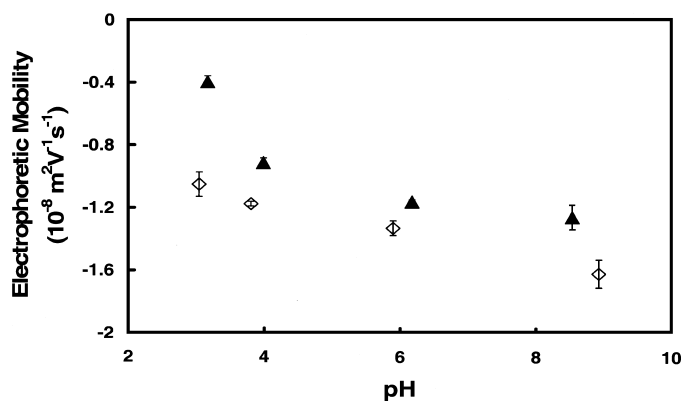


Figure 5. Zetapotential analysis of *B. subtilis* and *P. mendocina* as a function of pH. Closed triangles and open diamonds represent data points for *P. mendocina* and *B. subtilis* experiments, respectively.

Error bars represent standard error of three measurements.

DISCUSSION

The presence of the Fe-coating on the quartz grains significantly enhances the extent of bacterial adsorption onto the mineral surfaces for both *B. subtilis* and *P. mendocina* (Figures 2 and 3). The extent of bacterial adsorption onto uncoated quartz in these experiments is below the experimental limit of detection (i.e., within 2σ error of control experiments). Yee et al. (2000) also observed adsorption of *B. subtilis* to uncoated quartz to be below the limit of detection in similar experiments. The experiments in this study were all conducted at pH conditions significantly above the pH_{zpc} of the quartz surface (Stumm and Morgan 1981). The pH_{zpc} is the pH at which the net charge on a surface is zero. Therefore, under the experimental conditions, the quartz surface was negatively charged, as was the Fe surface above approximately pH 8. All of these results are consistent in that they each suggest that electrostatic interactions between the bacterial and mineral surfaces exert a controlling influence on the extent of adsorption. Bacterial adsorption occurs when the bacterial and mineral surfaces exhibit opposite surface charges, with decreasing adsorption as the surface charges change to be both negatively charged with increasing pH.

We use a surface complexation approach to model the bacterial adsorption data in order to relate pH and sorbent : sorbate ratio effects on adsorption to the speciation of the two surfaces involved. Our first step is to model the Fe-powder potentiometric titration data to determine site concentrations and stability constants for the important mineral surface species. One-site and two-site models were tested systematically with either single deprotonation reactions or double deprotonation reactions assigned to each site using FITEQL (Westall 1982)

as the computational tool. Because we measured bacterial adsorption at only one ionic strength, we neglect the effects of the surface electric fields on the adsorption equilibria. For each model tested, FITEQL calculates a variance function, $V(Y)$, that describes the goodness of fit of the model to the experimental measurements. The best-fitting model was determined to be the one that yields the lowest $V(Y)$ value and displays the best visual fit to experimental data. Each titration was modeled separately. A two-site model with two corresponding deprotonation reactions associated with each site was found to provide the best fit to the experimental data of all three Fe-powder titrations. An example of the model fit is illustrated for one of the data sets in Figure 4. This model is consistent with the findings of Dzombak and Morel (1990) whose models of Fe-mineral surfaces also suggest the presence of two reactive surface sites. We assume that the concentration of sites on the Fe-powder surface calculated by FITEQL can be scaled down to represent the concentration of sites on the lower surface area Fe-coated quartz surface through use of a scaling factor equal to the ratio of surface areas for the two minerals. We further assume that the Log K values determined for Fe-powder sites are equal to the Log K values for Fe sites on the Fe-coated quartz surface. The best fitting reaction stoichiometries with corresponding averaged deprotonation constants and averaged site concentrations for the Fe-coated quartz are given in Table 1.

With known values for the concentrations and acidity constants for the proton-active sites on each sorbing surface, we test the ability of a surface complexation model to account for the observed bacterial adsorption onto the Fe-coated quartz. Our

Table 1
Log K and site concentration values of *B. subtilis*, *P. mendocina*, and Fe-coated quartz surface sites

	[Site] ^a	Reaction	Log K
<i>B. subtilis</i> sites ^b	8.07×10^{-5}	$\text{R-A}_{(1)}\text{H}^0 \rightleftharpoons \text{R-A}_{(1)}^- + \text{H}^+$	-3.3
	1.12×10^{-4}	$\text{R-A}_{(2)}\text{H}^0 \rightleftharpoons \text{R-A}_{(2)}^- + \text{H}^+$	-4.8
	4.42×10^{-5}	$\text{R-A}_{(3)}\text{H}^0 \rightleftharpoons \text{R-A}_{(3)}^- + \text{H}^+$	-6.8
	7.37×10^{-5}	$\text{R-A}_{(4)}\text{H}^0 \rightleftharpoons \text{R-A}_{(4)}^- + \text{H}^+$	-9.1
<i>P. mendocina</i> sites ^c	9.58×10^{-5}	$\text{R-A}_{(1)}\text{H}^0 \rightleftharpoons \text{R-A}_{(1)}^- + \text{H}^+$	-3.4
	1.24×10^{-4}	$\text{R-A}_{(2)}\text{H}^0 \rightleftharpoons \text{R-A}_{(2)}^- + \text{H}^+$	-4.7
	5.08×10^{-5}	$\text{R-A}_{(3)}\text{H}^0 \rightleftharpoons \text{R-A}_{(3)}^- + \text{H}^+$	-6.5
	8.06×10^{-5}	$\text{R-A}_{(4)}\text{H}^0 \rightleftharpoons \text{R-A}_{(4)}^- + \text{H}^+$	-9.3
Fe-coated quartz sites	2.47×10^{-4}	$>\text{Fe-OH}_{(1)}^{\circ} - \text{H}^+ \rightleftharpoons >\text{Fe-O}_{(1)}^-$	-9.8
		$>\text{Fe-OH}_{(1)}^{\circ} + \text{H}^+ \rightleftharpoons >\text{Fe-OH}_{2(1)}^+$	-6.1
	2.00×10^{-4}	$>\text{Fe-OH}_{(2)}^{\circ} - \text{H}^+ \rightleftharpoons >\text{Fe-O}_{(2)}^-$	-8.1
		$>\text{Fe-OH}_{(2)}^{\circ} + \text{H}^+ \rightleftharpoons >\text{Fe-OH}_{2(2)}^+$	-4.3

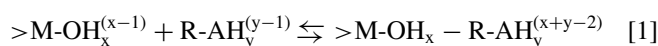
^aSite concentrations in moles/g.

^bData from Fein et al. (2004).

^cData from Borrok and Fein (2004).

electrophoretic mobility measurements indicate that the surfaces of both bacteria are negatively charged over the entire pH range studied. In addition, our titrations of the Fe-powder suggest that positive species are dominant on the Fe-surface in the pH range of maximum bacterial adsorption, with decreasing positive species concentrations and bacterial adsorption with increasing pH. The opposite charges of the bacterial and mineral surfaces, coupled with the correlation between positive mineral surface site speciation and extent of bacterial adsorption, suggests that electrostatic attraction between the bacterial and mineral surfaces controls the adsorption behavior in both bacterial systems studied. While the bacterial surface becomes more negatively charged with increasing pH, the concentration of positive mineral surface sites becomes significantly less dominant, as the concentration of neutrally and negatively charged mineral surface sites become significant. Therefore, the electrostatic attraction between sorbing surfaces begins to diminish with increasing pH, leading to a decrease in adsorption.

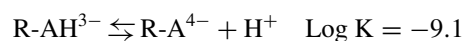
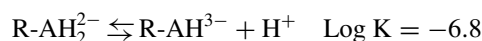
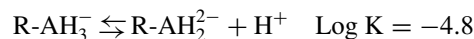
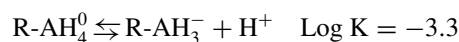
Yee et al. (2000) modeled adsorption of *B. subtilis* to corundum through single-site to single-site adsorption with the following generic reaction:



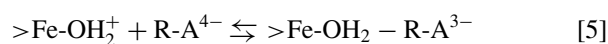
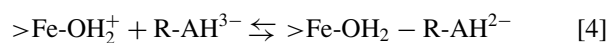
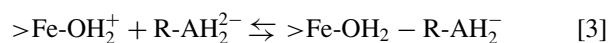
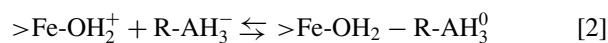
where, $>M-OH_x^{(x-1)}$ represents a mineral surface site of a metal oxide, R-A represents a bacterial surface functional group type, x can equal 0, 1, or 2, and y can have a value of either 0 or 1. In this study, all possible reaction stoichiometries described by reaction (1) were tested, but no single reaction could account for the adsorption of *B. subtilis* to Fe-coated quartz over the entire pH range. It is unlikely that the adsorption of bacteria to mineral surfaces is governed strictly by site-to-site interactions as reaction (1) implies. However, as pointed out by Yee et al. (2000), the use of site-specific modeling to account for bacterial adsorption to mineral surfaces can be used as a semi-empirical approach that can account for pH and bacteria: mineral ratios better than traditional partition coefficients.

The site-specific reaction approach can account for the effect of surface speciation on bacterial adsorption. The misfit of single-site specific adsorption models to experimental data implies that the summation of the electronegativity on the bacterial surface rather than a component from a single site controls adsorption. However, two simultaneous adsorption reactions involving different bacterial surface sites can not be considered because mass balance constraints can not be adequately imposed on such a system. Therefore, we have devised a semi-empirical approach that combines each of the four bacterial surface sites into a unified hypothetical site that undergoes four sequential deprotonation reactions. This approach enables us to account for the surface speciation (and hence the surface charge), while being able to impose a mass balance constraint on the total site concentration. The combined site has a concentration equal to the total site concentrations of each site on the bacterial surface, and undergoes successive deprotonation steps, with Log K

values equal to the discrete Log K values determined from the bacterial titration data. Thus, general deprotonation reactions and corresponding constants of the combined site for *B. subtilis* are as follows:



Under this formalism, the adsorption reactions involving interaction between the deprotonated form of these sites with the positively charged Fe-coated quartz surface can be expressed as:



Because we observed the most bacterial adsorption under low pH conditions, we first test the ability of reaction (2) alone to account for the observed adsorption behavior. We then included reactions (3) to (5), adding each one sequentially, to determine which model provides the best fit to the adsorption data. FITEQL 2.0 (Westall 1982) was used to determine the reaction stoichiometry and corresponding equilibrium constant that best describes the observed adsorption behavior.

Reaction (2) alone can not account for the pH dependence of adsorption that we observed. However, a model that includes reactions (2) to (5) yields an excellent fit to the pH dependence of the adsorption. The calculated log K values for reactions (2), (3), (4), and (5) for the best-fitting model are 4.4 ± 0.6 , 4.6 ± 0.6 , 4.0 ± 1.0 , and 5.9 ± 0.8 , respectively, and the model fit is represented as a dashed curve in Figure 2. The model that incorporates reactions (2) through (5) yields an excellent fit to the experimental data, providing a means of quantitatively accounting for the effect of pH on the extent of bacterial adsorption. A similar modeling approach could not be applied to the *P. mendocina* adsorption data due to the relatively large experimental uncertainties relative to the low extent of observed adsorption for that system.

We apply a similar cumulative site approach to model the effect of bacteria: mineral mass ratio on the extent of adsorption of *B. subtilis* onto the Fe-coated quartz. However, because the mass ratio experiments were conducted at a fixed pH (6.5) and hence fixed surface site speciation, the data do not constrain which bacterial surface sites are most important in affecting adsorption. Therefore, we model the adsorption data using the reaction that involves the bacterial surface site that is prevalent

under the pH conditions of the mass ratio experiments. That is, at the pH of the mass ratio experiments, the bacterial surface is dominated by the $R-AH_2^{2-}$ species, and we assume that reaction (3) controls the bacterial adsorption behavior. This modeling approach yields an excellent fit to the adsorption data, with a Log K value for reaction (3) of 4.5 ± 0.1 . $R-AH_2^{2-}$ is also the dominant reactive site on the *P. mendocina* surface at the pH of the mass ratio experiments involving that bacterial species. Using reaction (3) to model the *P. mendocina* bacteria : mineral mass ratio data provides an excellent fit to adsorption data, with a calculated Log K value of 3.8 ± 0.05 . The model fits to both *B. subtilis* and *P. mendocina* bacteria : mineral mass adsorption data are represented by dashed curves in Figure 1. The value from the *B. subtilis* mass ratio data is within the uncertainty of the Log K value of 4.6 obtained from the *B. subtilis* pH adsorption data model, indicating that the same set of thermodynamic equilibrium constants can successfully account for *B. subtilis* adsorption onto Fe-coated quartz as a function of both pH and bacteria : mineral mass ratio. Yee et al. (2000) also observed the same trend by showing that Log K values obtained for the adsorption of *B. subtilis* to corundum were similar between pH data and bacteria : mineral mass ratio data.

Although the adsorption of both *B. subtilis* and *P. mendocina* onto Fe-coated quartz is likely controlled by electrostatic attraction, the extent of *B. subtilis* adsorption is significantly greater than *P. mendocina* adsorption. The cause for the difference between the *B. subtilis* and *P. mendocina* behaviors may be due to differences in their cell wall structures. The outer surface of a gram-positive cell is characterized by a thick layer of peptidoglycan, rich in carboxylate and covalently bonded to encompass the entire outer surface of the cell. Secondary polymers, such as teichoic or teichuronic acids, are often present and introduce more functional groups into the peptidoglycan framework (Fortin et al. 1997). Conversely, the outer surface of a gram-negative cell consists of a thin layer of peptidoglycan overlain by an outer lipid/protein bilayer. Secondary polymers are not present in the peptidoglycan framework of a gram-negative cell, however the outer lipid/protein bilayer contains lipopolysaccharide (LPS) lipids (Fortin et al. 1997). Our electrophoretic mobility results indicate that *P. mendocina* has a lower surface charge than does *B. subtilis* and adsorption experiments are consistent with this.

CONCLUSION

Our experimental results demonstrate that electrostatic properties of both the bacterial and mineral surfaces control the extent of bacterial adsorption onto mineral surfaces in metabolically inactive systems. Neither *B. subtilis* nor *P. mendocina* exhibit significant adsorption onto the dominantly negatively charged quartz surface. Conversely, the presence of Fe-coatings on quartz surfaces significantly enhances the adsorption of bacteria, with enhancement occurring as a function of pH and bacteria : mineral mass ratio. The pH dependence of the data sug-

gests that adsorption is likely controlled by the relative electrostatic properties of both the bacterial and mineral surfaces. The gram-positive *B. subtilis* adsorbs onto Fe-coated quartz to a greater extent than does the gram-negative *P. mendocina*, with differences in adsorption behavior likely caused by differences in electrostatic properties and cell wall structure. Furthermore, this study shows that a semi-empirical cumulative site surface complexation model can be successfully applied to describe the adsorption of bacteria to mineral surfaces as a function of both pH and bacteria : mineral mass ratio by specifically accounting for the charge and speciation of sites on the sorbing surfaces. Our data illustrate that significant differences exist in the adsorption reaction equilibrium constants between bacterial species. However, this modeling approach offers a semi-empirical means of accounting for pH and mass ratio effects on bacterial adsorption onto mineral surfaces using a single set of equilibrium constants.

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