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Does metal adsorption onto bacterial surfaces inhibit or enhance aqueous metal transport? Column and batch reactor experiments on Cd–*Bacillus subtilis*–quartz systems

Nathan Yee *, Jeremy B. Fein

Department of Civil Engineering and Geological Sciences, University of Notre Dame, Notre Dame, IN 46556, USA

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Abstract

In this study, we investigate the effects of bacteria on metal transport through mineral-filled columns, and how the effect varies with pH and mineralogy. We measured *Bacillus subtilis* and aqueous Cd transport through quartz and Fe-coated quartz columns as a function of pH, using a separation technique to determine the absolute concentrations of aqueous and bacterially bound Cd in the effluent. Experimental results indicate that under certain conditions, bacteria enhance Cd transport through the columns. In these cases, the migration of Cd through the column is facilitated by Cd adsorption onto bacterial surfaces, and by transport of the bacteria. However, under other conditions, the transport of bacteria is inhibited, causing a retardation in Cd mobility. Under these conditions, the bacteria are immobile due to bacterial adsorption onto mineral surfaces and/or straining by the sand matrix. In separate experiments, we test whether a surface complexation modeling approach can be used to account for metal adsorption in complex systems such as these that contain both bacterial and mineral surfaces. We performed batch adsorption experiments with aqueous Cd, *B. subtilis*, and quartz, quantifying metal and bacterial adsorption as a function of pH. We use these experiments as a rigorous test and extension of the surface complexation approach to more realistic geologic systems than have been studied previously. The experimental results show that thermodynamic stability constants, determined from binary systems, can be used to successfully quantify the distribution of Cd between the aqueous phase and the bacterial and mineral surfaces, and can be used to estimate the distribution of mass in systems not directly studied in the laboratory. The column results indicate that modeling of contaminant transport in bacteria-bearing systems requires not only accurate flow models, but also chemical speciation models that quantify the role of bacterial adsorption under a range of subsurface conditions. Surface complexation modeling offers a means to account for the adsorption chemistry in these complex systems.

Keywords: Bacteria; Metal; Transport; Sorption; Surface complexation; Porous media; Quartz

1. Introduction

Bacteria are ubiquitous in near-surface geologic systems, with reported concentrations ranging from 10^4 to 10^8 cells/ml of fluid in groundwater aquifers, and 10^7 to 10^{10} cells/gram of solid in soil environments (Alexander, 1977; Ghiorse and Wilson, 1988). Aque-

* Corresponding author. Current address: The School of Earth Sciences, University of Leeds, Leeds LS2 9JT, UK. Tel.: +44-113-233-5203; fax: +44-113-233-5259.

E-mail address: nyee@earth.leeds.ac.uk (N. Yee).

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ous metal cations display a high affinity for bacterial surfaces (e.g., Beveridge and Murray, 1976, 1980; Harvey and Leckie, 1985; Gonç alves et al., 1987; Konhauser et al., 1993; Pons and Fuste, 1993; Fein et al., 1997; Fowle et al., 2000); hence, it is likely that the mobility of certain aqueous metal contaminants in groundwater systems is closely tied to that of the bacteria. Under certain hydrogeologic and geochemical conditions, bacteria are mobile in the subsurface, as has been observed by both laboratory and field studies (e.g., Allen and Morrison, 1973; McDowell-Boyer et al., 1986; Harvey et al., 1989; Gannon et al., 1991; Bengtsson and Lindquist, 1995; Johnson and Logan, 1996). Under other conditions, bacteria are immobile due to bacterial attachment onto mineral surfaces and/or bacterial straining by the rock matrix. If bacteria are mobile, then the transport of metal contaminants adsorbed onto bacteria cell walls can potentially be facilitated. Conversely, if bacteria are immobile, then bacteria–metal adsorption can potentially enhance retardation. However, the effect of bacterial adsorption on metal transport is unknown, and with our current knowledge, it is impossible to quantitatively assess the magnitude by which bacterial adsorption can enhance or retard the migration of metal contaminants.

Previous studies have shown that the presence of bacteria in soil suspensions can significantly affect metal mobility (Chanmugathas and Bollag, 1987a,b, 1988). However, the mobilization mechanisms are poorly understood, and it is unclear if metal adsorption onto bacterial surfaces plays an important role. The potential for bacterial adsorption to enhance contaminant transport has been discussed (McCarthy and Zachara, 1989), and Lindqvist and Enfield (1992) demonstrated that hydrophobic organic contaminants can adsorb onto bacterial surfaces, and that contaminant mobility can be enhanced by co-transport with bacterial cells. Laboratory column experiments have demonstrated that heavy metal transport can be facilitated by metal sorption onto a mobile inorganic colloid phase (e.g., Eichholz et al., 1982; Puls and Powell, 1992; Newman et al., 1993; Karathanasis, 1999). Column experiments by Saiers and Hornberger (1996) showed that kaolinite colloids enhanced the transport of cesium ions, and that the extent of enhancement was dependent on colloid concentration. Roy and Dzombak (1997) conducted Ni transport experiments with sand-packed laboratory columns, and demonstrated that the enhancement of Ni transport is controlled by the mobility of the colloid, and that the colloid deposition efficiency varies with pH and metal concentration. However, the surface reactivity of bacterial cells is significantly different from that of inorganic mineral colloids, and it is unknown if bacteria would exert similar effects on metal mobility.

The effect of bacterial adsorption on metal transport is controlled by the affinity of the metal to adsorb onto bacterial surfaces, and by the affinity of the bacterial cells to adsorb onto or be immobilized by the rock matrix. The mobility of bacteria in soil and aquifer systems is influenced by a variety of chemical and physical factors. Previous studies have shown that surface chemistry can control bacterial transport through porous media (Kinoshita et al., 1993; Jewett et al., 1995; Baygents et al., 1998), and that bacterial attachment is strongly influenced by mineralogy, solution pH, and ionic strength (van Loosdrecht et al., 1989; Scholl et al., 1990; Scholl and Harvey, 1992; Mills and DeJesus, 1994; Yee et al., 2000). Similarly, the affinity of metal to adsorb onto bacterial surfaces is also strongly dependent on surface speciation and solution chemistry (e.g., Plette et al., 1996; Fein et al., 1997; Daughney and Fein, 1998; Fowle and Fein, 1999). Therefore, in order to accurately quantify the effect of bacterial adsorption on metal transport, the effects of surface and solution chemistry must be taken into account.

Bulk partitioning models, such as Langmuir or Freundlich isotherms, are commonly used to quantify metal–bacteria adsorption (e.g., Harvey and Leckie, 1985; Mullen et al., 1989; McLean et al., 1990; Pons and Fuste, 1993; Ledin et al., 1996; He and Tebo, 1998), bacteria–mineral adsorption (e.g., Walker et al., 1989; Ohmura et al., 1993; Mills and DeJesus, 1994), and metal distribution in multi-sorbent systems (e.g., Turner et al., 1992; Ledin et al., 1999). Partitioning adsorption models are relatively easy to apply and do not require a detailed understanding of the surfaces or adsorption mechanisms. However, partition coefficients are system-specific, and are applicable only to the conditions (pH, fluid and/or mineralogical compositions) at which they were determined.

Surface complexation modeling is a quantitative means to estimate the amount of metal adsorbed onto geologic surfaces, including mineral (Davis and Kent,

1990) and bacterial surfaces (Plette et al., 1996; Fein et al., 1997; Daughney et al., 1998; Fowle and Fein, 1999; Pagnanelli et al., 2000; Haas et al., 2001; Yee and Fein, in press). Surface complexation modeling allows for predictive calculations over a wide range of conditions beyond those directly tested in the laboratory. The adsorption reactions that describe the formation of the bacterial surface complexes can be included as part of an overall network of chemical reactions, and in this way, metal partitioning behavior can be estimated over a wide range of pH and ionic strength conditions, and as a function of solute/sorbent ratios. Numerous studies have characterized the metal sorption behavior in binary metal–bacteria and metal–mineral systems; however, there is much less research focused on metal adsorption in mixed bacteria–mineral systems (e.g., Small et al., 1999). It is currently unknown if equilibrium constants determined in binary metal–bacteria and metal–mineral systems can be extended to account for adsorption in ternary metal–bacteria–mineral systems.

The objective of this study is to determine and quantify the range of potential effects that bacterial adsorption exerts on metal transport in bacteria–mineral–water systems. We perform column experiments to measure Cd and *Bacillus subtilis* transport through mineral-filled columns as a function of pH and mineralogy (quartz and Fe-coated quartz). The column transport experiments in this work extend previous research in three important ways: (1) metal transport experiments are conducted with bacteria, which represent a significantly different surface chemistry and reactivity than inorganic colloids, (2) we control and test the effects of pH and mineralogy, and demonstrate the ability of bacteria to enhance or retard metal transport under different chemical conditions, and (3) we measure both the aqueous and total concentration of metal in the effluent, explicitly accounting for the amount of metal associated with the bacterial cells. In this study, we also perform batch adsorption experiments to ascertain whether a surface complexation model can be used to quantify metal adsorption in a mixed bacteria–mineral system. Data from the column experiments provide insights into the role of bacterial adsorption on metal transport, and the batch experiments demonstrate the potential for using surface complexation theory to quantify these effects.

2. Materials and methods

2.1. Growth conditions

The bacteria species used in our experiments is *B. subtilis*, a gram-positive aerobic species commonly found in groundwater environments. Bacterial cells were initially cultured in 3 ml of trypticase soy broth with 0.5% yeast extract for 24 h at 32 °C, then transferred to 1 l of broth and grown for another 24 h, also with 0.5% yeast extract. The cells were removed from the nutrient medium by centrifugation, rinsed in 0.1 M $NaNO_3$ electrolyte solution (the electrolyte used in the experiments) and soaked in 0.03 M HNO_3 for 1 h to remove contaminant cations from the bacterial surface. The cells were then rinsed five more times with 0.1 M $NaNO_3$, and centrifuged for 60 min at 7500 rpm to determine their wet weight.

Previous work in this laboratory indicates that the wash procedure does not significantly alter the cell wall structure, nor does it cause bacterial sporulation. We performed viability studies and inspected the bacterial cells with scanning electron microscopy (SEM) after the acid treatment, and we found that the cells were both viable and intact, without significant quantities of extracellular polysaccharides on the cellular surface. All experiments were conducted in nutrient absent conditions; hence, the bacterial cells were not likely to have been actively metabolizing during the experiments.

2.2. Mineral preparation

The quartz mineral powder used in the batch experiments was obtained from Aesar Chemical, and the quartz sand used in the column experiments was industrial quartz sand obtained from Accusand. Both types of quartz were washed using 1 M NaOH, rinsed with ultrapure (18 MΩ) water, washed again with 10% HNO_3, then rinsed repeatedly with ultrapure water until the supernatant exhibited a constant pH approximately equal to the pH_{zpc}. The mineral powder was then dried at 60 °C for 48 h. The surface areas determined by BET isotherm analysis of the Aesar quartz mineral powder and the Accusand quartz sand were 0.2249 and 0.028 m^2/g, respectively.

Fe-coated quartz sand was used in some of the column experiments. Fe coating was obtained by add-

ing 10.36 g of $Fe(NO_3)_3$ to 101.16 g of the Accusand quartz sand in 1.0 l of ultrapure water. The mixture was continuously stirred while titrating with aliquots of 6 M NaOH until the solution pH reached 6.0. The coated grains were then rinsed with ultrapure water five times and oven-dried at 60 °C. The surface area determined by BET isotherm analysis of the Fe-coated sand was 0.064 m^2/g.

2.3. Column experiments

Column experiments were conducted with a Kontes glass column (1.0×10.0 cm, with a 20 μm frit), through which fluid flow was controlled with a peristaltic pump, and effluent was directly fed into a UV–VIS spectrophotometer. Ten grams of industrial quartz sand or Fe-coated quartz sand was added to the column using a wet packing procedure to obtain close packing and uniform porosity. The sand-filled column was then flushed with the electrolyte solution for 30 min. Control experiments were performed with a conservative tracer (red dye in ultrapure water) to measure the flow of a nonreactive solute. The hydrologic factors controlling flow were kept constant for all column experiments, and flow conditions and UV–VIS spectrophotometer readings were controlled and recorded automatically by computer.

Cd and Cd + bacteria breakthrough experiments were performed by first preparing a 200 ml electrolyte input solution with a known amount of metal and bacteria. Metal transport experiments were conducted with $10^{-5.1}$ mol/l Cd (~ 1 ppm), and metal + bacteria experiments were conducted with $10^{-5.1}$ mol/l Cd and 1.0 g/l bacteria (wet weight). Cd was added to the input solution from a concentrated, low pH, 1000 ppm aqueous Cd standard. The input solution was adjusted to the desired pH, and was allowed to equilibrate for 1 h prior to introduction to the column. For the experiments conducted at near-neutral pH, a 2[*N*-morpholino]ethanesulfonic acid (MES) buffer was added to the solution (to a concentration of 10^{-3} molal) to maintain constant pH for the duration of the experiment. After equilibration, the input solution was pumped through the column at a constant rate of 3.5×10^{-2} ml/s. The effluent solution from the column was pumped through the UV–VIS spectrophotometer to measure bacterial concentration. The UV–VIS spectrophotometer sampled the effluent solution at a wavelength of 400 nm, every 30 s, using the measurement approach described below.

At approximately 2-min intervals, 4 ml of solution was collected at the outlet of the spectrophotometer. The final solution pH was measured in each sample, and the samples were analyzed for aqueous Cd (Cd_{aq}) and total Cd (Cd_T) concentrations. The Cd_{aq} concentrations were determined by filtering the sample through a 0.45-μm filter, and analyzing the filtrate for Cd. The Cd_T concentration is defined as the Cd dissolved in solution (Cd_{aq}) plus the Cd adsorbed onto the bacterial surface (Cd_{ads}). For each sample, Cd_T was determined by first desorbing the Cd from the bacterial cells in the sample. Desorption was accomplished by adding a 20 μl aliquot of concentrated HNO_3 acid to each sample, driving the solution pH to ~ 1.7. As demonstrated by Fowle and Fein (2000), reversibility of metal adsorption onto bacterial cell walls is total and rapid under these conditions. The acidified samples were allowed to equilibrate for 1 h, then filtered and analyzed for aqueous Cd. All aqueous Cd analyses were conducted using an inductively coupled plasma-atomic emission spectroscopy technique measuring emission wavelengths of 214.433 and 226.502 nm. The Cd standards used for calibration were prepared using the same electrolyte used in the experiments, and analytical uncertainty was determined to be approximately ± 3%. Control experiments were performed by sampling the input solution prior to its introduction onto the column and performing the desorption procedure. These experiments demonstrated full recovery of Cd through the desorption technique.

2.4. Batch experiments

Batch experiments were conducted to measure Cd and *B. subtilis* adsorption in a mixed Cd–*B. subtilis*–quartz system. Experiments were performed as a function of pH and as a function of Cd and bacterial concentrations. A known wet weight of bacteria was suspended in a Cd-bearing 10-ml volume of 0.1 M $NaNO_3$, and placed in test tubes in contact with a known weight of quartz mineral powder. The pH of each test tube was adjusted to the desired value using 0.1 M NaOH or HNO_3, and was allowed to equilibrate for 2 h. During equilibration, the test tubes were placed on a rotating rack that stirred the tubes end over end 20 times/min. After equilibration, the final solution pH

was measured. Replicate experiments were performed to measure Cd and *B. subtilis* adsorption onto the quartz grains separately.

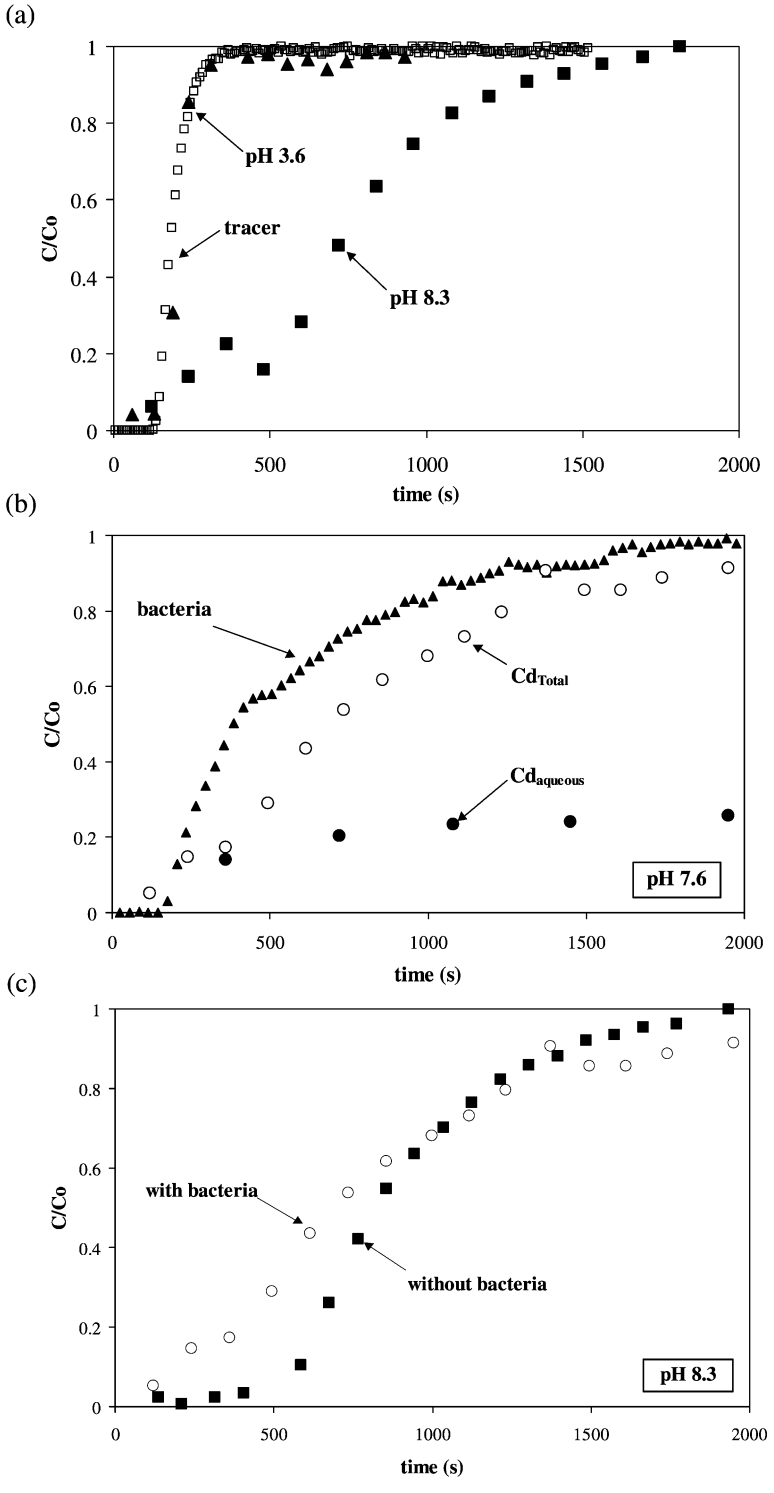
Bacterial attachment was measured by analyzing the concentration of unattached bacterial cells in each batch experiment, and subtracting this concentration from the known total concentration of bacteria in each system (Yee et al., 2000). Separation of the unattached bacteria from the sample fraction that contained quartz powder and attached bacteria was accomplished by injecting 3 ml of a sucrose solution (60% by weight) into the bottom of the mineral–bacteria suspension. Because the sucrose solution is denser than the sample fraction that contains the bacteria suspended in solution, but is less dense than the mineral grains (including those with bacteria attached), the sucrose separates the sample fractions according to density. The mineral powder with any adsorbed bacteria sink to the bottom of the test tube, and the unadsorbed bacteria and aqueous solution float on top of the sucrose layer. After the sucrose separation, the unattached bacteria fraction was extracted with a pipette. The extent of bacterial adsorption was determined by measuring the unattached bacterial concentration using a UV–VIS spectrophotometric technique. The samples were analyzed at a wavelength of 400 nm, and the concentration of bacteria was determined by comparing the sample to a calibration curve computed from five standards of known bacteria concentrations of *B. subtilis*. A wavelength of 400 nm could resolve low concentrations of bacteria and yielded a linear calibration curve. Control experiments were performed without the mineral present to determine whether adsorption to the test tubes occurred, and to quantify the efficiency of the separation technique. The control experiments demonstrated that 9% of the bacteria were lost during the separation procedure. This amount of bacteria loss did not vary as a function of pH from pH 3 to 10. Hence, 9% was subtracted from each experiment to account for separation efficiency. The experimental uncertainties of the bacterial concentration measurement, determined by the reproducibility of the experiments, were $\pm$ 10%.

The extent of Cd adsorption was determined by measuring the concentration of aqueous metal remaining in solution after the equilibration time. Samples were filtered (0.45 μm), acidified to prevent precipitation, and analyzed for dissolved Cd using the ICP–AES technique described above. Any decrease in dissolved Cd concentration that occurred during the experiment was assumed to be caused by Cd adsorption onto the bacterial cell wall and/or mineral surface. Previous control experiments in our laboratory have demonstrated that Cd adsorption onto the experimental apparatus is negligible.

3. Results

Cd and *B. subtilis* breakthrough curves from the column experiments conducted with cleaned quartz sand are depicted in Fig. 1. The effluent concentrations of the inert tracer, of Cd, and of bacteria are normalized to the input concentrations of each, C/C_0, and the data are plotted as a function of time. The effluent tracer is a nonreactive solute and represents the average flow velocity of water passing through the column. The conservative tracer breakthrough curve is controlled by the hydrologic/physical properties of the sand matrix, and does not vary with changes in solution chemistry. Therefore, the breakthrough of the effluent tracer is the same for all column experiments conducted in this study. The Cd breakthrough curve from the experiments conducted without bacteria is illustrated in Fig. 1a. The solid triangles represent Cd breakthrough at pH 3.6, and the solid squares show the Cd breakthrough at pH 8.3. At low pH, the Cd breakthrough curve is virtually identical to that of the conservative tracer (open squares), while at high pH, there is a strong retardation effect, indicating that the Cd transport through the column is pH-dependent. At high pH, Cd is reacting with the quartz surface, and the adsorption reaction retards the transport of Cd through the mineral-filled column. At the Cd concentrations used in these experiments, the hydrolysis and precipitation of Cd does not occur until above pH 9.5 (Baes and Mesmer, 1976). Therefore, at both pH values (3.6 and 8.3), the only significant aqueous Cd species is Cd^{2+}.

Fig. 1b illustrates the results from the quartz column experiment conducted with an input solution containing both Cd and bacteria at pH 7.6. The results, shown in terms of Cd_{aq} (solid circles), Cd_T (open circles) and *B. subtilis* (open triangles) concentrations, indicate that the addition of bacteria to the system significantly reduces the concentration of Cd_{aq}


(a)
C/Co
time (s)
pH 3.6
tracer
pH 8.3
(b)
C/Co
time (s)
bacteria
Cd_Total
Cd_aqueous
pH 7.6
(c)
C/Co
time (s)
with bacteria
without bacteria
pH 8.3

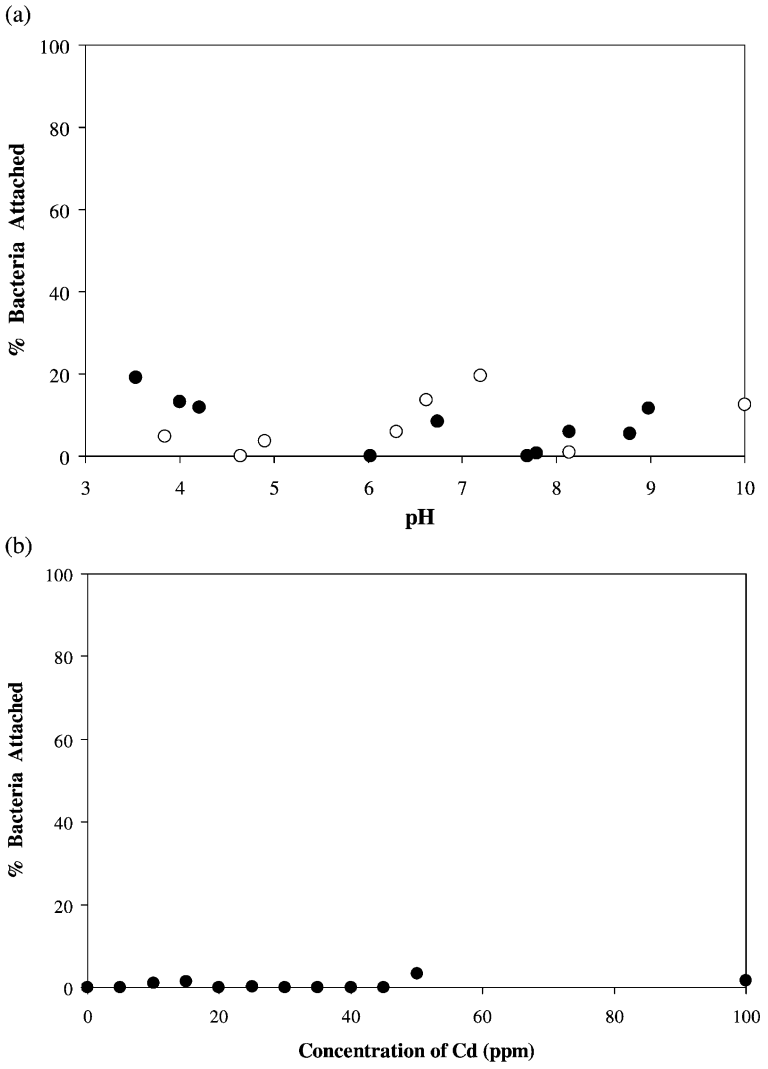


Fig. 2. Percentage of *B. subtilis* (1.0 g/l) attached to the quartz mineral powder (100 g/l) (a) as a function of pH with 10.4 ppm Cd (●) and without Cd (○), (b) as a function of Cd concentration at constant pH 5.2.

in the effluent solution. The breakthrough concentration of Cd_T is significantly higher than that of Cd_{aq}, indicating that a large fraction of Cd_T is associated with the bacterial cells. The output concentration of bacteria eventually reaches $C/C_0 = 1.0$, indicating that the bacteria are mobile and are readily transported through the column. The data displayed in Fig. 1b show that the Cd_T breakthrough curve closely follows the *B. subtilis* breakthrough trend, suggesting that the Cd_T transport is facilitated by the presence of bacteria. Similarly, Fig. 1c displays Cd breakthrough curves for experiments conducted at pH 8.3, either with bacteria (open circles) or without bacteria (solid squares), and the results indicate that the presence of bacteria en-

Fig. 1. Cd (input [Cd] = 8.76×10^{-5} molal) breakthrough from the quartz column as a function of time, (a) without bacteria at pH 3.6 (▲), at pH 8.3 (■), and conservative tracer (□), (b) at pH 7.6 with 1.0 g/l bacteria, (○) total Cd, (●) free Cd and (△) *B. subtilis*, (c) at pH 8.3 ± 0.3 with 1.0 g/l bacteria (○), and without bacteria (■).

hances the initial Cd breakthrough and accelerates Cd transport. Column transport experiments conducted at pH 8.3 showed that at 500 s, the amount of Cd_T transported through the column with bacteria was approximately 0.3 C/C_0, whereas Cd_T without bacteria was approximately 0.05 C/C_0. The presence of bacteria enhanced the transport the initial Cd breakthrough by a factor of 6. Batch adsorption experiments were conducted to measure *B. subtilis* attachment onto the quartz. The results indicate that *B. subtilis* displays a very low affinity for the quartz surface over a wide pH range (Fig. 2a), and that high concentrations of Cd do

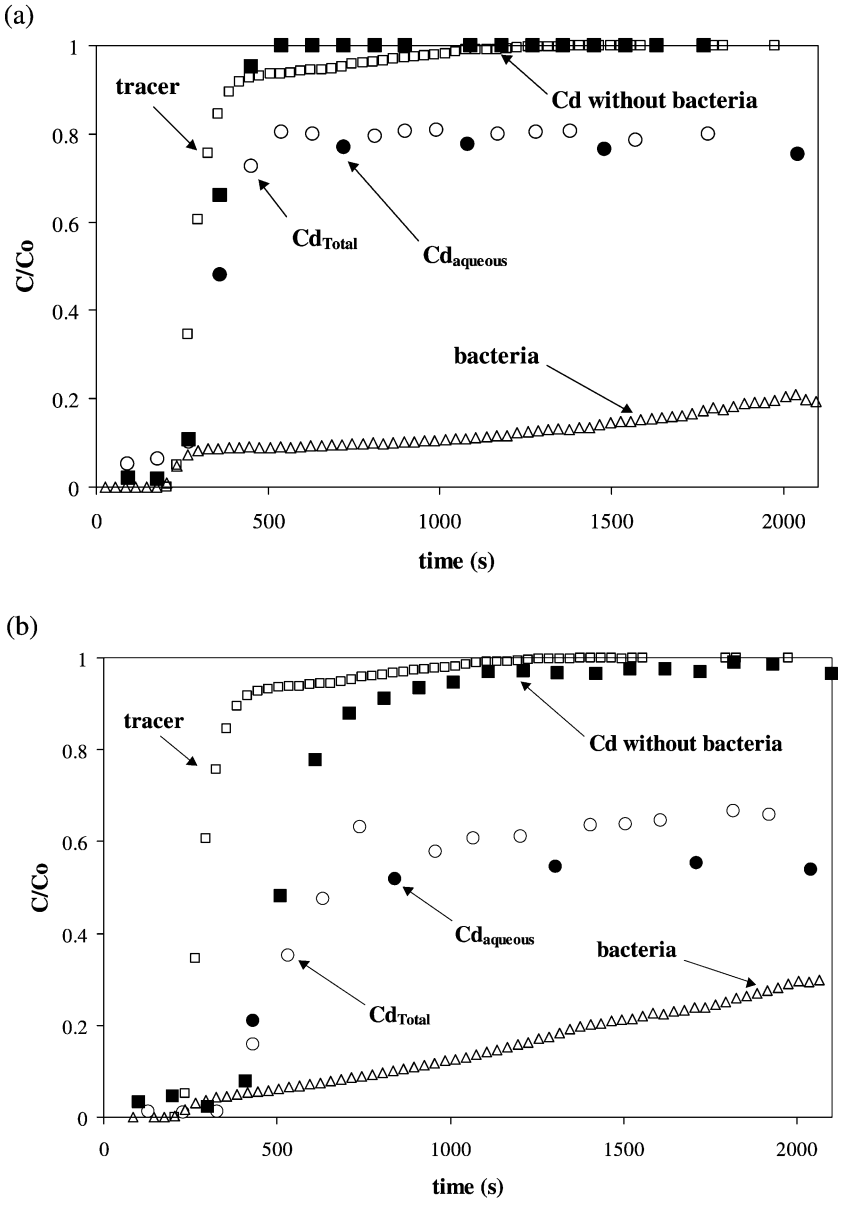


Fig. 3. Cadmium ($[Cd]_T = 8.76 \times 10^{-5}$ mol/l) breakthrough, Fe-coated quartz column as a function of time, (□) conservative tracer, (■) total Cd without bacteria, (○) total Cd with 1.0 g/l bacteria, (●) free Cd with bacteria, and (△) *B. subtilis*, (a) pH 5.7 and (b) pH 6.6.

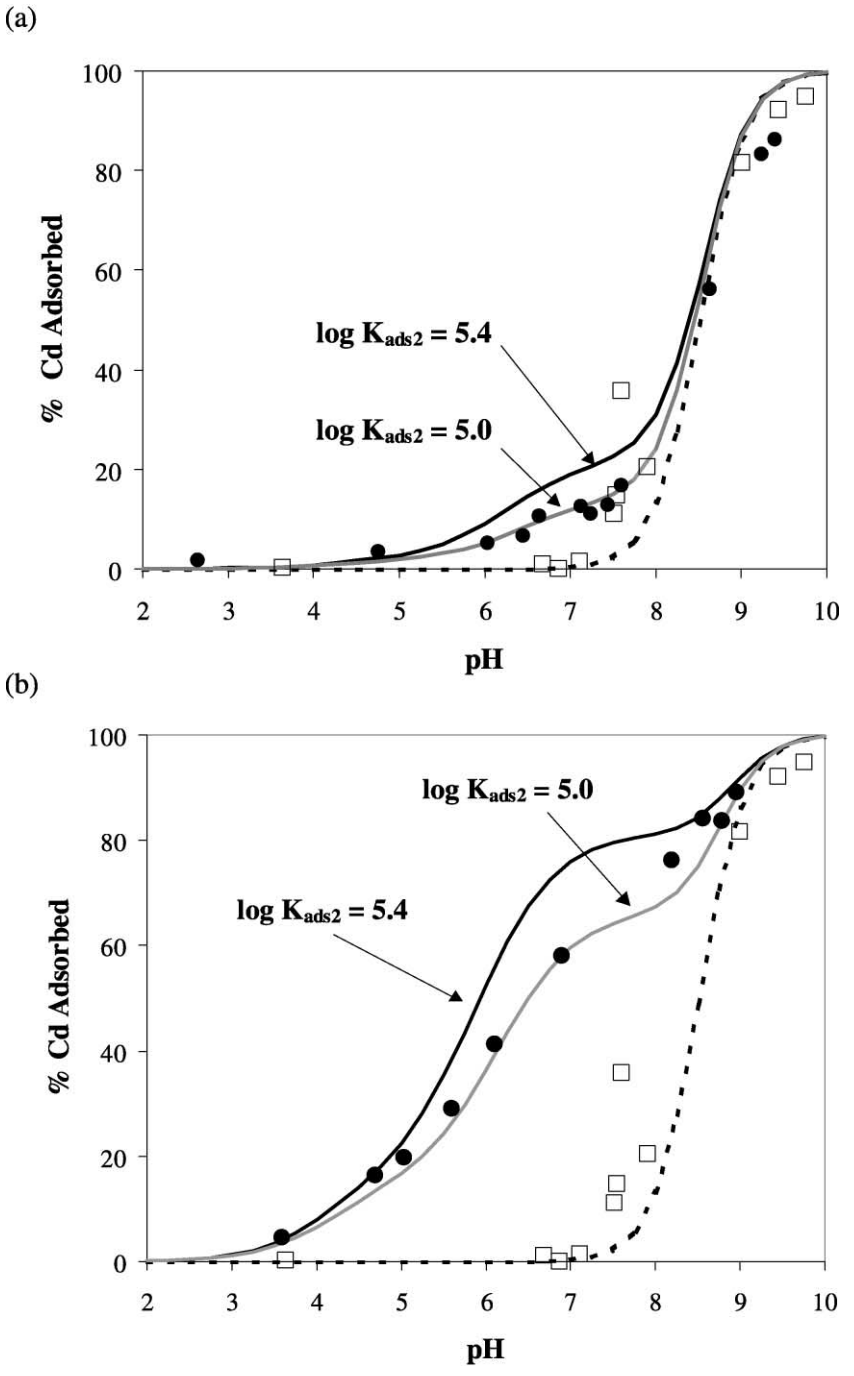


Fig. 4. Percentage Cd ($[Cd]_T = 8.76 \times 10^{-5}$ mol/l) adsorbed as a function of pH with 100 g/l of quartz and (a) 0.1 g/l bacteria (▲) and (b) 1.0 g/l bacteria (●). The open squares (□) represent Cd–quartz adsorption data (without the presence of bacteria), and dashed line is an independent model prediction of Cd adsorption onto the quartz surface alone. The solid lines represent independent model predictions of Cd adsorption onto both the mineral and bacterial surfaces, the black line with a log K_{ads2} value of 5.0, and the grey solid line with a log K_{ads2} value of 5.4.

not enhance the extent of attachment (Fig. 2b). Therefore, bacteria are effectively inert in the quartz sand column, and the transport of bacteria through the column is unaffected by the presence of Cd in the system.

The column experiments conducted with Fe-coated quartz grains display markedly different behavior, with the presence of bacteria retarding Cd transport (Fig. 3). Fig. 3a illustrates the results of Fe-coated quartz grain column experiments conducted at pH 5.7. For these experiments, we measured the column breakthrough of the conservative tracer (open squares), a Cd solution without bacteria (solid squares), Cd_T and Cd_{aq} with bacteria (circles), and the bacteria breakthrough for the bacteria-bearing experiment (triangles). The experiments with and without bacteria have similar Cd breakthrough edges, but the experiment containing bacteria reaches a plateau at approximately $C/C_0 = 0.8$. The breakthrough of bacteria is significantly reduced, indicating that to some extent, the bacterial cells are immobilized in the column. The identical experiment was repeated at a higher pH of 6.6 (Fig. 3b), and an increase in the retardation of Cd was observed. Cd breakthrough in the pH 6.6 experiment containing bacteria reached a plateau at $C/C_0 = 0.6$. The concentrations of Cd_{aq} in both experiments shown in Fig. 3 are only slightly lower than the Cd_T concentrations, indicating that the Cd_T is largely composed of aqueous Cd, and a small percentage of the total Cd was bound to mobile bacterial cells. The breakthrough of bacterial cells is markedly lower than that observed in the quartz grain column experiments, although slightly higher bacterial concentrations were observed in the pH 6.6, relative to the pH 5.7 Fe-coated quartz experiments. Fig. 4 depicts the measured adsorption of Cd onto *B. subtilis* and quartz mixtures at two different bacterial concentrations. Fig. 4a,b illustrates Cd adsorption onto quartz with 0.1 and 1.0 g bacteria/l, respectively (based upon an average bacterial weight of 10^{-12} g/cell, these bacteria concentrations correspond to 10^8 and 10^9 bacteria per milliliter of fluid). The data indicate that the adsorption of Cd onto *B. subtilis* and quartz is strongly pH-dependent. At low pH, Cd adsorption onto the bacteria and mineral surfaces does not readily occur. With increasing pH, the surface functional groups on the bacteria and mineral surfaces deprotonate and react with the aqueous Cd. At 0.1 g bacteria/l (Fig. 4a), a slight increase in Cd adsorption is observed from pH 4.5 to 7.5, which is likely due to bacterial adsorption. Above pH 8, a significant increase in adsorption occurs. Control experiments were conducted with the Cd solution only and with Cd–quartz without bacteria. The results of the control experiments show that Cd does not precipitate under the experimental conditions below pH 9 (data not shown). In Fig. 4, the Cd–quartz adsorption data are represented as open squares, and an independent model prediction of Cd adsorption onto the quartz surface is depicted by the dashed line. A comparison of the Cd–quartz and the Cd–quartz–bacteria adsorption data in Fig. 4a suggests that the Cd adsorption behavior at low bacterial concentrations (0.1 g bacteria/l) is controlled by the quartz mineral surface. Cd adsorption experiments conducted with quartz and 1.0 g bacteria/l (Fig. 4b) show a significant shift in the adsorption edge. Cd adsorption occurs under relatively low pH conditions, with adsorption increasing with increasing pH, and a large fraction of the Cd is adsorbed under neutral pH conditions. In near-neutral to slightly acidic conditions, we observed a significant enhancement in Cd adsorption with increasing bacterial concentration, suggesting that the metal preferentially adsorbs onto the bacterial surfaces under these conditions.

4. Discussion

4.1. Column experiments

The column experiments demonstrate that bacteria can significantly affect Cd mobility, and that the extent to which bacteria facilitate or retard Cd transport is strongly dependent on mineralogy and solution pH. The quartz column experiments conducted without bacteria (Fig. 1a) indicate that at low pH, Cd behaves like a conservative tracer. The Cd remains dominantly in the aqueous phase, and exhibits negligible interaction with the solid quartz matrix. Conversely, at high pH, we observed a strong Cd retardation effect in the bacteria-free system, indicating that at least some of the Cd adsorbed onto the quartz surface. Under both pH conditions, Cd^{2+} is the dominant aqueous species, and these results are consistent with the pH dependence that we observed in the batch experiments for Cd^{2+} adsorption onto the quartz surface (Fig. 4).

When bacteria are added to the system, the breakthrough of aqueous Cd is significantly retarded, but that of total Cd is enhanced (Fig. 1b,c). Because Cd adsorbs onto bacterial cell walls, the presence of bacteria changes the distribution of Cd in the experimental system. In Fig. 1b, the dominant fraction of the Cd_T breakthrough is Cd_{ads}. The *B. subtilis* and the Cd_T breakthrough curves are similar, suggesting that the mobility of the bacteria controls the overall transport of the metal. Experiments conducted with and without bacteria (Fig. 1c) demonstrate that the presence of bacteria facilitates the initial Cd breakthrough. In the absence of bacteria, the metal is adsorbed onto the mineral surface and Cd retardation occurs. However, in the presence of bacteria, the metal adsorbs onto the bacterial surface, and because the bacteria are mobile, they enhance the transport of Cd through the column.

The mobility of bacteria in the column experiments is largely controlled by bacterial attachment onto mineral surfaces. Bacterial attachment in nature is governed by an initial adsorption process, followed by subsequent metabolic processes such as biofilm formation and exudate production (Little et al., 1997). Because the column experiments were conducted at nutrient absent conditions, and because the duration of the experiments is on the order of minutes, we have eliminated metabolic adhesion effects from the experiments and physicochemical adsorption is likely to be the dominant attachment process. The results from *B. subtilis*–quartz batch adsorption experiments indicate that the attachment of *B. subtilis* onto quartz is weak, and that the extent of attachment does not change over a wide pH range (Yee et al., 2000). Both surfaces are negatively charged between pH 3 and 10, and the lack of attachment is likely caused by electrostatic repulsion between the negatively charged quartz surface and the negatively charged bacterial cell wall (Yee et al., 2000). The *B. subtilis*–quartz batch adsorption experiments conducted in the presence of aqueous Cd (Fig. 2) demonstrate that the presence of even high concentrations of the aqueous metal cation does not significantly enhance the bacteria–mineral interaction. The data suggest that cation bridging between the two surfaces does not occur, and that the formation of a ternary, *B. subtilis*–Cd–quartz complex, is not significant.

Our results indicate that bacterial transport through a mineral filled column is strongly dependent on the mineralogy of the solid matrix. Previous studies have shown that bacteria display a high affinity for Fe–oxide surfaces (Mills and DeJesus, 1994; Glasauer et al., 2000). Similarly, Yee et al. (2000) show that the adsorption of *B. subtilis* cells onto an Al-oxide surface is highly pH dependent. Significant bacterial adsorption onto the Al-oxide surface occurs under low pH conditions, with decreasing adsorption with increasing pH from pH 3 to 7. Due to the chemical and crystallographic similarities between Fe- and Al-oxides, we expect Fe-oxides to exhibit a similar bacterial adsorption behavior.

The results displayed in Fig. 4 indicate that only a small fraction of the bacteria is transported through the Fe-coated quartz column, suggesting that the attachment of bacterial cells onto mineral grains inhibits bacterial transport. It is also possible that at low pH, bacterial coagulation and enhanced bacteria matrix straining diminishes bacterial transport. However, at the pH conditions studied, this is not likely to be a significant effect. The Fe-oxide coating does not markedly change the size of the mineral grains, and hence the expected porosity and permeability of the column should be roughly similar between the quartz and the Fe-oxide-coated quartz experiments. At pH 5.7 (Fig. 3a), the transport of bacteria through the Fe-coated quartz column reaches approximately 0.2 C/C_0 at 2000 s. A significant fraction of the bacteria is immobilized in the column, and therefore the fraction of Cd that is adsorbed onto the bacterial cells is also immobilized. A comparison of the Cd breakthrough with and without bacteria indicates that the presence of bacteria reduces the concentration of Cd measured in the effluent solution. The breakthrough of Cd_{aq} is virtually identical to Cd_T, indicating that little or no Cd_{ads} is transported through the column.

A comparison of the pH 5.7 (Fig. 4a) and the pH 6.6 (Fig. 4b) data shows that the distribution of Cd between the aqueous phase and solid phase (adsorbed onto mineral or bacterial surfaces) is sensitive to solution pH. We observe a decrease in the effluent Cd concentrations in the pH 6.6 experiment relative to the experiment conducted at pH 5.7. The retardation of Cd is enhanced under the higher pH conditions because Cd adsorption onto the relatively immobile bacteria increases with increasing pH (e.g., Fein et al., 1997). The magnitude of this retardation effect is dependent on the tendency of the aqueous metal cat-

ion to adsorb onto bacterial surfaces, and on the mobility of the bacteria. Similar to the case for the pH 5.7 experiment, the Cd_{aq} in the pH 6.6 experiment is virtually identical to Cd_T during the early stages of the experiment (until approximately 1000 s), indicating that little or no Cd_{ads} is transported through the column. However, after approximately 1000 s, Cd_{aq} concentrations become increasingly lower than those of Cd_T, most likely due to the increased breakthrough of significant and increasing concentrations of bacteria, with associated Cd_{ads} on their surfaces.

The column experiments demonstrate the potential range of effects that bacteria can exert on contaminant transport in the subsurface. Under some conditions (e.g., Fig. 1b), bacteria are highly mobile and can enhance aqueous metal cation mobilities. Under other conditions (e.g., Fig. 2c), bacterial adhesion and/or matrix straining makes the bacteria immobile, so that adsorption of the aqueous metal cation onto the bacterial surface significantly diminishes metal transport. These experiments indicate that quantitative modeling of the effects of bacteria on contaminant transport must be flexible enough to account for these changing effects. Surface complexation modeling provides a means for doing so, accounting for changes in solution chemistry and/or matrix mineralogy on the extent of adsorption.

4.2. Surface complexation theory

Surface complexation modeling is advantageous to partitioning approaches in that it explicitly accounts for the adsorption reactions that occur in the system of interest (e.g., Bethke and Brady, 2000; Koretsky, 2000). Here we describe the surface complexation framework that we use to quantitatively model adsorption in our experimental systems. Bacterial adsorption reactions involve the bacterial cell wall (Beveridge and Murray, 1976), which, in the case of *B. subtilis*, is composed primarily of peptidoglycan, a linear polymer chain containing sugars and amino acids, and teichoic acid, a large molecule composed of repeating units of sugars and phosphates (Beveridge, 1989). The cell wall of *B. subtilis* is neutrally charged at pH values less than 2.2, and becomes increasingly negatively charged with increasing pH (Harden and Harris, 1952) due to the successive deprotonation of organic functional groups on the cell wall surface. Potentiometric titrations have shown that there are three types of proton-active surface functional groups on the cell wall of *B. subtilis* (Fein et al., 1997). Bioassays have determined the identity of the dominant organic functional groups to be carboxyl, phosphoryl and hydroxyl groups (Beveridge and Murray, 1980). The deprotonation reactions of these groups can be described by the following equilibria:

$$R-COOH^0 \Leftrightarrow R-COO^- + H^+ \quad (1)$$

$$R-POOH^0 \Leftrightarrow R-PO_4^- + H^+ \quad (2)$$

$$R-OH^0 \Leftrightarrow R-O^- + H^+ \quad (3)$$

where R denotes the bacterium to which each functional group type is attached. The distribution of protonated and deprotonated sites on the cell surface can be quantified with the corresponding mass action equations:

$$K_1 = \frac{[R-COO^-][H^+]}{[R-COOH^0]} \quad (4)$$

$$K_2 = \frac{[R-PO_4^-][H^+]}{[R-PO_4H^0]} \quad (5)$$

$$K_3 = \frac{[R-O^-][H^+]}{[R-OH^0]} \quad (6)$$

where the brackets represent either the concentration of bacterial surface species in moles of sites per liter or the molal activities of aqueous species. The negative log acidity constants (pK_a) for these reactions are 4.82 ± 0.14, 6.9 ± 0.5, and 9.4 ± 0.6, respectively (Fein et al., 1997). The adsorption of Cd^{2+} onto a deprotonated carboxyl and phosphoryl surface site can be described with the reaction:

$$Cd^{2+} + R-COO^- \Leftrightarrow R-COO(Cd)^+ \quad (7)$$

$$Cd^{2+} + R-PO_4^- \Leftrightarrow R-PO_4(Cd)^+ \quad (8)$$

Cd^{2+} partitioning between the solid and aqueous phase is, therefore, quantified with the corresponding mass action equations:

$$K_{ads1} = \frac{[R - COO(Cd)^+]}{[Cd^{2+}][R - COO^-]} \quad (9)$$

$$K_{ads2} = \frac{[R - PO_4(Cd)^+]}{[Cd^{2+}][R - PO_4^-]} \quad (10)$$

Fein et al. (1997) report values of 3.4 and 5.4 for log K_{ads1} and log K_{ads2}, respectively, a surface area of 140 m^2/g, and carboxyl and phosphoryl site concentrations of 1.2×10^{-4} and 4.4×10^{-5} mol/g.

We define the standard state for aqueous species as a hypothetical 1 molal solution that exhibits the behavior of infinite dilution at 25 °C and 1 bar. Departures from this standard state for aqueous ions are quantified using the Davies equation for activity coefficients. Activity coefficients for neutral aqueous species are assumed to be unity. The standard state for both bacterial and mineral surface complexes is a hypothetical 1 molal site at 25 °C and 1 bar, referenced to zero surface potential. Deviations from this standard state are caused by electrostatic interactions between ions and the bacterial surface and are accounted for with the Boltzmann accumulation factor (Stumm and Morgan, 1996):

$$K_{apparent} = K_{intrinsic} \exp(-\Delta Z F \Psi / RT) \quad (11)$$

where F and R are Faraday's constant and the gas constant, respectively, T is absolute temperature, $K_{intrinsic}$ represents the equilibrium constant of the adsorption reaction, referenced to zero surface charge, ΔZ is the change in the charge of the surface species in the reaction of interest, and Ψ is the electric field potential associated with the bacterial surface. To account for the electrostatic surface effects of the bacterial cell wall, we use a constant capacitance model for the electric field and a capacitance value of 8.0 F/m^2 (Fein et al., 1997).

The quartz surface speciation can be modeled with $>Si-OH_2^+$, $>Si-OH^0$, and $>Si-O^-$ as the stoichiometries for the surface sites in contact with pure water (Stumm and Morgan, 1996). In this model, the surface acidity is quantified through the following equilibria:

$$> Si - OH_2^+ \Leftrightarrow > Si - OH^0 + H^+ \quad (12)$$

$$> Si - OH^0 \Leftrightarrow > Si - O^- + H^+ \quad (13)$$

where >Si represents a crystallographically bound silicon atom at the mineral surface. The pK_a values for reactions (12) and (13) are -2.4 and 8.4, respectively (Sverjensky and Sahai, 1996), yielding a pH_{zpc} of 3.0. Cd adsorption onto the quartz surface can be described by the reaction:

$$Cd^{2+} + > SiOH \Leftrightarrow > SiO(Cd)^+ + H^+ \quad (14)$$

with associated mass action equation:

$$K_{ads2} = \frac{[> SiO(Cd)^+][H^+]}{[Cd^{2+}][> SiOH]} \quad (15)$$

Schindler et al. (1976) report a log K_{ads3} value of -6.09, a surface site concentration of 9.68×10^{-6} mol/m^2, and a capacitance value of 1.1 F/m^2.

4.3. Batch adsorption modeling

We use the batch adsorption experiments on the ternary Cd–*B. subtilis*–quartz system to test whether surface complexation modeling can account for the observed distribution of Cd, based on equilibrium constants determined from binary systems. Because ternary Cd–*B. subtilis*–quartz complexes are negligible, we can apply the stability constants determined in binary Cd–quartz and Cd–*B. subtilis* systems to describe the Cd partitioning behavior in this ternary system. We use the surface speciation program FITEXP (J. Lützenkirchen, personal communication) and Eqs. (1)–(15) to calculate independent model predictions for Cd adsorption onto the mixed bacteria and mineral surfaces at 0.1 and 1.0 g bacteria/l. FITEXP is a modified version of the chemical equilibrium program FITEQL 2.0 (Westall, 1982), and can simultaneously account for the surface electric field of both the bacterial and mineral surfaces. We invoke a constant capacitance model to describe the electric double layers associated with the bacterial cell wall and quartz mineral surface.

The modeling exercise indicates that a surface complexation approach can successfully account for the range of metal adsorption behaviors possible in ternary metal–bacteria–mineral systems. The calculated distribution of Cd between the aqueous phase, the mineral surface, and the bacterial surface is depicted in Fig. 4. At 0.1 g bacteria/l, the model predicts a small amount of Cd adsorption onto the bacterial surface from pH 4 to 7. This system represents a quartz-dominated regime in which the bacterial concentration is low, and therefore the extent of adsorption is small in the mid-pH range. Above pH 8, the model predicts a significant increase in adsorption due to Cd adsorption onto the quartz mineral surface. At 1.0 g bacteria/l (a bacteria-dominated system), the predicted extent of Cd adsorption from pH 4 to 8.5 is much larger than that predicted for the 0.1 g bacteria/l system, with predicted Cd adsorption increasing markedly from pH 3 to 7. The calculated adsorption curve begins to plateau at pH 7, but increases again above pH 8, due to Cd adsorption onto the quartz surface. For both cases considered here, the extent of adsorption and the pH dependency of adsorption agree very well with the experimental data. The calculations suggest that much of the adsorption behavior in the 1.0 g bacteria/l system is controlled by the bacterial surface, and that the adsorption observed in the 0.1 g bacteria/l system dominantly involves the mineral surface. The general agreement between the independent model predictions and the observed extents of adsorption provides strong evidence that surface complexation modeling can successfully account for the overall adsorption behavior as well as for the distribution of metal between the bacteria and the quartz surfaces in a wide range of multi-sorbent systems.

The model slightly overpredicts the extent of observed adsorption from approximately pH 5.5 to 8, and is likely due to uncertainties in the stability constant value for the bacterial surface Cd–phosphoryl complex, which dominates the adsorption behavior in this pH range. In general, metal–carboxyl stability constant values are known more precisely than those of metal–phosphoryl complexes. There are relatively few data points that constrain the value of the Cd–phosphoryl stability constant (Fein et al., 1997). However, the discrepancies between the predicted and observed adsorption behaviors can be accounted for by decreasing the Cd–phosphoryl stability constant ($\log K_{ads2}$) from 5.4 to 5.0 (as depicted in Fig. 4), a difference that is within the uncertainty of the original experimentally determined value. Clearly, the accuracy of surface complexation models depends on accurate and precise values for the equilibrium constants for the important reactions in each system of interest. However, the modeling in Fig. 4 shows that even with the relatively imprecise Cd–phosphoryl stability constant value that we currently have, the surface complexation approach can successfully account for adsorption behavior and Cd distribution in the experimental systems.

4.4. Application to column transport experiments

In the column experiments, both fluid flow and adsorption chemistry combine to control mass transfer through the columns. We can use a surface complexation modeling approach to gain insights into the adsorption chemistry of the systems, and to quantify the distribution of Cd between the aqueous solution, and the mineral and bacterial surfaces. The only column experiments to which such a modeling approach can be applied currently are the Cd–*B. subtilis*–quartz experiments (Fig. 1b). Because the column experiments were designed to qualitatively demonstrate bacterial effects on aqueous metal cation transport, we did not measure stability constants for the metal and bacterial surface complexes with the Fe-oxide. In addition, we did not measure surface coverage percentage of the Fe-oxide on the quartz grains. Therefore, it is impossible to quantitatively model the Fe-oxide column experiments using a surface complexation approach.

The best test of the approach is the Cd–*B. subtilis*–quartz column transport experiment depicted in Fig. 1b, in which significant Cd adsorption onto the bacterial surface occurs. The column experiment was conducted using 1 g/l bacteria and $10^{-5.1}$ M Cd in the input solution. At the pH of the experiments (pH 7.6), the surface complexation model predicts that a total of approximately 80% of the Cd is removed from solution due to adsorption, and approximately 20% remains as Cd_{aq} (see solid curve in Fig. 4b). The Cd_{aq} measured in the column effluent is consistent with this independent model prediction of Cd distribution, with $C/C_0 = 0.2–0.25$. The model further predicts that under these conditions, less than 1% of the total Cd is adsorbed onto the quartz surface, and that the

dominant sorbent is the bacterial surface (79.6% of the total Cd is adsorbed onto the bacterial cell wall). These model predictions are consistent with the results of the column experiments, which show that the dominant fraction of Cd is adsorbed onto the bacteria cells. Our results indicate that surface complexation modeling can successfully estimate the distribution of Cd in multi-sorbent systems. Surface complexation modeling provides a method for quantifying the adsorption chemistry that occurs in subsurface systems in which system parameters such as solution pH, solution composition, and matrix mineralogy change as a function of time or space.

5. Conclusions

Our column experiments show that in bacteria-bearing systems, the fate and mobility of metal contaminants can be closely tied to that of the bacteria. In the quartz column experiments, the bacteria are mobile, and they enhance Cd transport through the column. However, in the Fe-coated quartz column, the bacteria are immobile and they retard metal transport. Therefore, under certain conditions, bacteria adsorption enhances metal transport, and under other conditions, bacterial adsorption retard metal transport. The column experiments demonstrate the range of potential bacterial effects, and underscore the need for a flexible modeling approach for quantifying contaminant transport.

The batch adsorption experiments demonstrate that the surface complexation approach can be used successfully to quantify the adsorption of Cd in a mixed *B. subtilis*–quartz system as functions of both pH and solute/sorbent ratios. In systems with noninteractive sorbents, binary metal–bacteria and metal–mineral equilibrium constants can be used to calculate the distribution of metal in ternary metal–bacteria–mineral systems. The advantage of the surface complexation approach is that stability constants do not vary with solution or system compositions, and therefore can be used to estimate the distribution of mass in systems not directly studied in the laboratory.

Quantifying metal–bacteria–mineral adsorption reactions is a crucial step toward predicting when enhanced transport or retardation of metal contaminants due to bacterial adsorption will occur, and how sizeable an effect will ensue in either case. Metal–bacteria–mineral interactions are strongly dependent on surface and solution chemistry. This results presented in this study demonstrate that the modeling of contaminant transport in bacteria-bearing systems requires not only accurate fluid flow models, but also chemical speciation models that can quantify the role of bacterial adsorption under a wide range of subsurface conditions.

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