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THE THERMODYNAMICS OF METAL-BACTERIA
INTERACTIONS

A Dissertation

Submitted to the Graduate School
of the University of Notre Dame
In Partial Fulfillment of the Requirements
for the Degree of

Doctor of Philosophy

by

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Notre Dame, Indiana
November, 1999
THE THERMODYNAMICS OF METAL–BACTERIA INTERACTIONS

Abstract

by

David A. Fowle

Identifying and quantifying the controls on metal mobilities in weathering environments is critical in order to understand processes such as global element cycling, mass transport in near-surface water-rock systems, and sedimentary diagenesis. Bacteria are ubiquitous in low temperature geologic systems, and numerous laboratory and field studies demonstrate that bacteria can facilitate the formation and dissolution of minerals, and enhance or inhibit metal transport through adsorption reactions. However, despite the growing evidence that bacteria play a key role in many geologic processes in low temperature systems, our understanding of the rates and mechanisms of bacterial effects remains rudimentary.

The reversibility of metal-bacteria adsorption reactions were studied utilizing batch desorption and pH-stat experiments. The observed extent of desorption in the experimental systems is in excellent agreement with the amount estimated from a surface complexation model based on independently conducted adsorption experiments. Competitive cation adsorption experiments were conducted in experimental systems containing one or two bacterial species. In all cases studied, the
estimated adsorption behavior is in excellent agreement with the observations, with only slight differences that were within the uncertainties of the estimation and experimental procedures. The adsorption of the uranyl ion to *Bacillus subtilis* was studied as function of time, pH, and solid:solute ratio. The U adsorption data require two separate adsorption reactions, with the uranyl ion forming surface complexes with the neutral phosphate functional groups and the deprotonated carboxyl functional groups of the bacterial cell wall.

A systematic study of the effects of bacteria cell walls on the extent of mineral precipitation is also described. The results indicate that bacterial cell walls can not induce precipitation at under-saturated mineral conditions. These experiments unequivocally constrain the effects non-metabolizing bacteria have on mineral precipitation, and are in disagreement with previous unconstrained experiments.

The results presented within this dissertation demonstrate that bacteria are likely to have a profound effect on the mobility of metal cations in many near-surface geological systems, and that these interactions can be constrained through the use of equilibrium thermodynamics.
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CHAPTER 1

INTRODUCTION

The mobilities of metal cations in low temperature geologic settings must be determined to accurately model geochemical processes such as weathering and global element cycling, and to effectively guide remediation efforts of contaminated groundwater. Both physical and chemical processes control the mobility of metal pollutants in groundwater aquifers. The physical processes include diffusion, advection, dispersion, and colloidal phase transport (Freeze and Cherry, 1979). The mobility of an aquifer contaminant is also controlled through its interactions with other dissolved constituents and with the aquifer materials (i.e., chemical processes). The majority of the research on chemical controls on metal mobility has focused on the effects of solution chemistry and aquifer mineralogy on metal transport. However, recent field investigations of these systems provide evidence that much of the exposed mineral surfaces (and therefore surface area that is exposed to fluid flow) are coated with attached microorganisms or biofilms. Therefore, our current inability to quantify
the effects of bacteria on metal mobilities under a variety of system conditions precludes the incorporation of the effects of microorganisms in metal transport models. Previous studies have established the effects of aqueous complexation and humic and fulvic acids on metal adsorption (Murphy and Zachara, 1995; Langmuir, 1997), yet similar investigations into the quantitative effects of bacteria are lacking. The focus of this dissertation was to develop a thermodynamic framework for the description of metal-bacteria adsorption/desorption and precipitation reactions. The results from this study not only further our understanding of bacteria-water-rock interactions, but they will enable the inclusion of bacterial effects into quantitative geochemical models of metal transport in near-surface hydrologic systems.

Bacteria are among the oldest forms of life on earth and are ubiquitous in low temperature fluid-rock systems. In geologic systems, bacteria have been found to have cell concentrations ranging from $10^6$-$10^{10}$ cells/cm$^3$ (Albrechtsen and Winding, 1992). In fact, bacteria can thrive, with numbers reaching $10^5$ microbes/g of rock (McLean et al., 1996), even in harsh environments such as deep sea hydrothermal vents, hot springs, and deep sedimentary aquifers (Cowen, 1989; Ghiorse and Wilson, 1988; McLean and Beveridge, 1990). The cell walls of bacteria display a high affinity for metal cations, primarily because bacteria rely on diffusion for the incorporation of nutrients. Within the polymers of the cell wall is a repeating series of organic functional groups predominantly carboxyl, phosphoryl, amino and hydroxyl (Beveridge and Murray, 1976, 1980; Beveridge et al. 1982; Beveridge, 1988; Beveridge, 1989). These functional groups adsorb metal cations effectively, and have been postulated to protect the cell from metal toxicity, and to act as a nutrient storage
repository (Beveridge, 1989). Similar to organic acids, the cell wall functional groups protonate and deprotonate in response to changing solution pH. Deprotonation causes the overall surface charge of the bacteria to become increasingly negative with increasing pH, thus increasing the tendency for aqueous cations to bind to the surface (Harden and Harris, 1953; Collins and Stotzky, 1992). In many cases it has been found that bacteria can accumulate metal in excess to the mass of the bacteria itself (Macaskie, 1986).

Near-surface or surface waters, whether contaminated or not, typically contain numerous aqueous metal ions and a variety of mineralogical and biological surfaces. Figure 1.1 depicts a model of the complexity of these types of dynamic systems on a molecular scale. To accurately model such a complex system on this mechanistic scale one must couple models of redox chemistry, aqueous complexation, surface complexation, and chemical kinetics. Many of the chemical reactions displayed in Figure 1.1 have been modeled successfully within the framework of equilibrium thermodynamics, and can be described by unique stability constants that predict the extent the reaction will proceed under a specific set of chemical conditions (Martell and Smith, 1977; Langmuir, 1997). The stability constants for these reactions are determined in single contaminant, single ligand or substrate experimental systems and can be subsequently combined with other stability constants to predict the chemical speciation of the contaminant in groundwater. The resulting chemical equilibrium
Figure 1.1.

Molecular scale processes affecting the mobility of metal cations in near-surface low temperature geological systems.
model can be coupled to physical models of fluid flow to predict the fate of the contaminant in groundwater.

However, few studies have attempted to provide a quantitative and mechanistic understanding of metal-bacteria adsorption and precipitation reactions. To date only three studies have invoked a site-specific metal-bacteria surface complexation model to describe these interactions (Fein et al., 1997; Daughney et al., 1998; Daughney and Fein, 1998). Similar to the stability constants for aqueous complexation, surface complexation chemical equilibrium theory offers a means to use experimental results from separate, isolated simple systems to estimate the distribution of mass (both dissolved and adsorbed) in more complex, multi-component systems such as those depicted in Figure 1.1. Geochemical equilibrium modeling enables recombination of these basic component reactions so that the equilibrium state can be determined for any system of interest. The application of surface complexation modeling is in its infancy, and the accuracy of the calculated equilibrium states is rarely determined through direct comparison with studied systems. Furthermore, no study to date has unequivocally demonstrated that the adsorption of metal ions onto the surface of bacteria is an equilibrium process, or that the results of single metal, single bacteria experiments can successfully be extrapolated to more complex systems. Fein et al. (1997) and Daughney et al. (1998) demonstrated the reversibility of the protonation of surface functional groups. However, the studies did not investigate the reversibility of metal adsorption reactions, nor the effects of competition for the bacterial surface functional groups. If contaminant transport models are to incorporate the surface complexation model to
describe metal-bacteria interactions, the reversibility of these reactions must be established. Furthermore, it is imperative that the ability of the model to cope with multi-contaminant, multi-surface situations be tested as a first step in its application to low temperature aqueous systems.

This dissertation thesis focuses on the determination of stability constants for selected adsorption reactions between calcium, copper, lead, and uranium and the gram positive soil bacteria *Bacillus subtilis* and *Bacillus licheniformis*. Copper, cadmium, lead, and uranium are considered toxic and are listed as priority pollutants by the Environmental Protection Agency (Keith and Telliard, 1979). Metals may be introduced to surface waters and groundwater aquifers through the smelting and refining of metal ores as well as subsequent runoff from mine tailings and waste rock piles. Other sources include the many industries that generate products such as batteries and plastics (Alloway, 1995). The burning of fossil fuels also can lead to atmospheric deposition of heavy metals on terrestrial and aquatic ecosystems (Alloway, 1995). The toxic effects of these heavy metals and uranium to humans or other mammals generally involve the accumulation of the metal or radionuclide in the kidneys or liver, ultimately leading to renal or liver failure and/or cancer. The toxic effects of these contaminants have been studied extensively and are well-documented (Fergusson, 1990).

The power of the surface complexation approach is that one can utilize the stability constants determined in this study, as well as those determined previously by Fein et al. (1997) and by Daughney and Fein (1998) to predict the fate of Cu, Cd, and Pb in complex metal-bacteria experiments, to estimate the extents of desorption of Ca
and Cd in reversibility experiments, and to separate adsorption and precipitation of Cu in controlled Cu-bacteria precipitation studies. A study similar to Fein et al. (1997) is also undertaken to provide the first mechanistic insights into the adsorption behavior between uranium and *Bacillus subtilis*.

Chapters 2 and 3 of this dissertation present experimental studies that test the assumptions inherent to the surface complexation approach: the reversibility of metal-bacteria adsorption reactions and the ability to utilize the stability constants to account for adsorption in complex multiple metal-bacteria systems. The reversibility of metal-bacteria interactions (Chapter 2) was studied by comparing estimated extents of desorption based on surface complexation modeling, to those observed in the experimental adsorption/desorption systems. The experiments also determine if extended adsorption contact time affects desorption kinetics. The experiments involved Ca and Cd adsorption/desorption onto the surface of a gram positive bacterium: *Bacillus subtilis*. Three types of experiments were performed: 1) Ca and Cd desorption from the cell wall of *B. subtilis* after 1 hour of adsorption contact; 2) Cd and Ca desorption from the cell wall of *B. subtilis* after >15 hours of adsorption contact; and 3) Ca and Cd desorption as a function of pH after 1 hour of adsorption contact.

A study of the competitive adsorption of metal cations onto two gram positive bacteria is described in Chapter 3. The objective of this study was to test the ability of a surface complexation approach to account for metal-bacteria interactions in complex near surface fluid-rock systems. A series of experiments are described that measure the extent of adsorption in mixed metal, mixed bacteria systems. This study
tests the surface complexation approach by comparing estimated extents of adsorption based on surface complexation modeling using stability constants derived from simple systems, to those we observed in the complex experimental systems. The batch adsorption experiments involved Ca, Cd, Cu, and Pb adsorption onto the surfaces of two gram positive bacteria: *Bacillus subtilis* and *Bacillus licheniformis*. Three types of experiments are described: 1) Single metal (Ca, Cu, Pb) adsorption onto a mixture of *B. licheniformis* and *B. subtilis*; 2) mixed metal (Cd, Cu, and Pb; Ca and Cd) adsorption onto either *B. subtilis* or *B. licheniformis*; and 3) mixed or single metal adsorption onto *B. subtilis* and *B. licheniformis*. Independent of the experimental results, and based on the site specific stability constants for Ca, Cd, Cu, and Pb interactions with the carboxyl and phosphate sites on *B. licheniformis* and *B. subtilis* determined by Fein et al. (1997), by Daughney et al. (1998), and in this study, we estimate the extent of adsorption that is expected in the above experimental systems.

The studies of Fein et al. (1997) and Daughney et al. (1998) provided stability constants for the binding of several heavy metals but do not investigate the adsorption behavior of radionuclides. The aqueous geochemistry of radionuclides is extremely complex and is dependent on aqueous complexation, surface complexation, redox chemistry and the solubility of many unique secondary radionuclide mineral phases. Uranium is utilized as a model radionuclide for this initial study of bacterial surface complexation (Chapter 4). Uranyl adsorption onto the gram positive soil bacterium *Bacillus subtilis* was measured using batch experiments in 0.1 M NaClO₄ as a function of pH, time, and solid:solute ratio at 25 °C. The experimental data were interpreted using a surface complexation approach, and the measurements constrain
the stoichiometry and thermodynamic stabilities of the important uranyl-surface complexes.

Chapter 5 describes a series of experiments that determine the effects that non-metabolizing bacterial cells have on the precipitation of Cu(OH)$_2$$_{\text{(S)}}$. There are two processes that may occur at the bacteria-water interface that influence metal distributions: surface adsorption and surface-induced mineral precipitation. Reversible adsorption of metal cations onto bacterial surfaces is documented in Chapter 2, and previous work by our group demonstrates that surface complexation modeling can accurately account for the metal adsorption (Fein et al., 1997). To date there have been no systematic studies of the effects of non-metabolic bacteria on the extent of mineral precipitation. Several studies have demonstrated that bacteria can influence the composition and morphology of minerals precipitating from aqueous solutions (Fortin and Ferris, 1998; Warren and Ferris, 1998) as well as the rate of precipitation from oversaturated solutions. It is often implied in field studies of biomineralization that bacteria not only enhance precipitation rates, but that they can cause precipitation at otherwise undersaturated conditions. Despite these claims, there have been no studies that unequivocally demonstrate this phenomenon. The objective of this study was to conduct experiments in which the solution chemistry was well-constrained so that we could explicitly account for adsorption and bulk solution saturation state, thereby isolating the bacterial effects on precipitation.

In order to define and quantify the role of bacteria in the formation of metal (hydr)oxides, we measured Cu adsorption and precipitation onto the surface of Bacillus subtilis as a function of pH, aqueous Cu activity, and time. To differentiate
between the adsorption and precipitation of Cu onto the cell walls of *B. subtilis* we utilize surface complexation modeling, control experiments, and equilibrium solubility measurements. Because we can explicitly account for Cu adsorption, and because the control experiments clearly define the saturation concentrations, these experiments unequivocally constrain the conditions at which mineral precipitation occurs.

There are many geochemical processes that control mass transport in aqueous systems as depicted in Figure 1.1. Many of these processes involve bacteria, and yet our understanding of the mechanisms and rates of reaction for such processes is rudimentary. Therefore the objective of this thesis is to examine and quantify several of these geochemical processes through a series of constrained laboratory experiments. This thesis represents an important step in the development and application of surface complexation models to bacteria-bearing water-rock systems.
References


CHAPTER 2

EXPERIMENTAL MEASUREMENTS OF THE REVERSIBILITY OF METAL-
BACTERIA ADSORPTION REACTIONS

2.1. Introduction

Chemical equilibrium models offer a means for estimating the adsorption/desorption behavior of aqueous metals in water-rock systems. A requirement for the application of these models to a geologic system is that the adsorption/desorption reactions that occur in the system must be fully reversible over the time scale of interest (Davis and Kent, 1990; Langmuir, 1997). Surface complexation models are a specific type of chemical equilibrium approach that can be used to quantify the extent of adsorption by explicitly describing the chemical reactions that occur between the solute and specific sites on the surface of interest (Stumm and Morgan, 1996). Geochemists typically use experiments to isolate a
specific metal-surface adsorption reaction and to determine an equilibrium constant for that reaction, making an implicit, but often unproven, assumption that equilibrium exists and that the surface adsorption reactions are fully reversible. However, few adsorption studies rigorously demonstrate the attainment of equilibrium, or effectively determine the kinetics or extent of desorption. If desorption is studied, the experiments generally promote complete solute desorption by drastically changing system conditions, such as lowering the solution pH to 2 or lower (Harvey and Leckie, 1985; Mamaril et al., 1997), or introducing a strong chelating agent such as EDTA (Puranik et al., 1995). A more sensitive test of reversibility is to measure the extent of desorption under conditions where only partial desorption occurs, comparing the extents of adsorption and desorption under identical pH and solution conditions.

Although some studies have tested the reversibility of adsorption/desorption reactions between metal cations and mineral surfaces (Padmanabham, 1983; Comans, 1987; Aharoni et al., 1992; Rybica et al., 1995), few studies have focused on the reversibility of metal-bacteria adsorption reactions. Bacteria are present in a wide variety of near-surface geologic settings (Kerr, 1997). In many of these systems, viable bacterial cells, dead cells, and cell wall fragments represent a large proportion of the surface area exposed to water. Numerous laboratory and field studies have demonstrated that bacteria effectively bind metal ions through adsorption reactions with the functional groups of the bacterial cell wall (Beveridge and Murray, 1976, 1980; Gonçalves et al., 1990; Konhauser et al., 1993; Fein et al, 1997; Fowle and Fein, 1999). Therefore, bacteria have the potential to affect the mass transport of aqueous metal ions through adsorption reactions in water-rock systems (McCarthy
and Zachara, 1989; Albrechtsen and Winding, 1992), making it crucial to accurately and quantitatively model bacteria-metal adsorption reactions.

The adsorption and desorption of Group I and II metals and trace metals onto mineral surfaces has been studied extensively. For example, Comans (1987) demonstrated, through a series of batch desorption experiments, that the reversibility of Cd adsorption onto illite requires a period of 7-8 weeks for complete desorption. In these experiments, the kinetics of adsorption of Cd onto illite were faster than the corresponding desorption reactions. Padmanabham (1983) compared the adsorption and the desorption behavior of Cu, Co, Zn, and Pb on goethite and demonstrated hysterisis between the adsorption and desorption reactions. Although these results are not directly applicable to the study of bacterial adsorption reactions, these studies demonstrate that equilibrium does not always exist for adsorption reactions in geologic systems. In order to determine if a chemical equilibrium approach can be applied to model a water-rock system, it is crucial to experimentally verify the existence of an equilibrium state by quantifying the extent of reversibility and the kinetics of the reactions.

A number of metal desorption studies involving bacteria have been conducted. Flemming et al. (1990) examined the remobilization of metals adsorbed to bacterial cell wall-clay composites. Cell wall-clay composites were incubated with metal solutions of Ag(I), Cu(II), and Cr(III) at near neutral pH for 15 minutes. Remobilization was initiated with the addition of a solution of nitric acid (to promote proton competition for surface sites), Ca (to promote ion exchange), or EDTA or fulvic acids (organic complexation). Flemming et al. (1990) found a significant
extent of remobilization in each of the experiments, indicating that aqueous speciation could significantly affect the stability of surface species. However, because Flemming et al. (1990) made no attempt to quantify the results with a chemical equilibrium model, and because they did not directly compare the desorption results to adsorption experiments conducted under identical conditions, full reversibility was not demonstrated.

Harvey and Leckie (1985) investigated the adsorption/desorption interactions of Pb with the surface of a gram negative marine bacterium. The experiments demonstrated that significant desorption of Pb occurs in response to a decrease in pH (from 7 to 2). Harvey and Leckie (1985) also found a correlation between the contact time of adsorption and the kinetics of desorption. If Pb was allowed to adsorb for 24 hours rather than 10 minutes, desorption kinetics were slower. However, Harvey and Leckie (1985) did not demonstrate reversibility because they did not compare their experimental results to a predicted extent of adsorption at pH 2.

Several other studies have also investigated the reversibility behavior of metal adsorption reactions with bacteria cell walls (Urrutia and Beveridge, 1993; Puranik et al, 1995; Manmaril et al., 1997). However, like the studies by Flemming et al. (1990) and by Harvey and Leckie (1985), none of these studies has rigorously tested whether full reversibility (and hence, attainment of an equilibrium state) of adsorption/desorption reactions occurs. In order to demonstrate equilibrium and reversibility, experiments must show that the concentration of metal on the bacterial surface is dependent only on system conditions (pH, solution composition, etc.), and
does not depend on whether that concentration was attained through adsorption or desorption.

The objective of this study is to test whether the adsorption of metal cations onto the surface of a gram positive bacterium is fully reversible. We do this in two ways: 1) by directly comparing the concentrations of metal on the bacterial surface attained through adsorption and through desorption under identical final system conditions; and 2) by comparing the concentration of metal on the bacterial surface attained through desorption to that estimated based on surface complexation models constructed from independently conducted adsorption experiments only. These latter experiments were conducted under a wide range of pH conditions. We use the common gram positive soil bacterium \textit{B. subtilis} in our study because its cell wall has been well characterized through biological assays (Beveridge and Murray, 1976), acid-base titrations, and metal adsorption studies (Fein et al., 1997; Daughney and Fein, 1998; Fowle and Fein, 1999). The two metal cations used in the study were chosen because they represent trace (Cd) and major (Ca) groundwater constituents and their adsorption behavior with \textit{B. subtilis} has been studied previously (Fein et al., 1997; Fowle and Fein, 1999). The experiments in this study not only test the reversibility of adsorption/desorption reactions (and hence the applicability of chemical equilibrium models in the first place), but they also determine the accuracy of the surface complexation approach in quantifying the distribution of aqueous metals in bacteria-bearing aqueous systems. Furthermore, we also test the effect of increasing adsorption contact time on the kinetics and extents of desorption. Results from our previous studies demonstrate that the protonation and deprotonation of the
cell wall functional groups is a fully reversible process (Daughney et al., 1998; Daughney and Fein, 1998). This study will provide an in depth study of desorption kinetics and determine if adsorption contact time affects the reversibility of metal-bacteria adsorption reactions.

2.2. Experimental Procedures

2.2.1. Bacteria Growth Procedures

The bacterial species \textit{B. subtilis} was prepared and cultured following the procedure outlined in Fein et al. (1997), and Daughney et al. (1998), except that the bacteria were washed in 1 mM EDTA for only 1 hour, and the electrolyte used was 0.1M NaClO$_4$. All solutions in this study were prepared with 18 M\(\Omega\) water. The wash protocol ensures that the cell walls of the bacteria are stripped of competing metals and anions. Without a thorough wash, the total concentration of metals in the system would be unknown, and the equilibrium states could not be calculated because the mass balance constraints would be unknown. Prior to each experiment, the bacteria were pelleted by centrifugation at 7500 rpm for 60 min. The mass of the pellet was measured in order to determine the concentration of surface functional groups in each experiment. Note that the weight of bacteria used in each experiment is not reported as a dried weight, but as a wet weight after centrifugation (to normalize the surface site concentration).
2.2.2. Metal Adsorption Experiments

Adsorption experiments were conducted at pH 4, using Teflon 250 mL reaction vessels. Washed bacteria were suspended in 0.1M NaClO₄ electrolyte, and 1000 ppm aqueous metal standard (Ca or Cd) was added to the bacteria-electrolyte solution to create a homogeneous parent solution of known bacterial and metal concentrations. The pH of the suspension in each vessel was adjusted to the desired pH value using less then 100μL of 2% HNO₃ or 0.5M NaOH. The pH of the reaction vessels was maintained through the use of an autotitrator in pH-stat mode. Samples were taken at predetermined time intervals, and each sample was filtered through a 0.45 μm nylon filter. Each filtrate was acidified and analyzed for aqueous metal content by flame atomic absorption spectroscopy (AAS).

2.2.3. Metal Desorption Kinetics Experiments

Desorption experiments were conducted at pH 4, using Teflon 250 mL reaction vessels. Washed bacteria were suspended in 0.1M NaClO₄ electrolyte, and 1000 ppm aqueous metal standard (Ca or Cd) was added to the bacteria-electrolyte solution to create a homogeneous parent solution of known bacterial and metal concentrations. Experiments involved a period of adsorption contact (1, 15, or 17 hours) at pH 8, followed by a desorption step, initiated by lowering the solution pH to 4. Nearly all of the dissolved metal was expected to adsorb onto the bacterial surface at pH 8. Between a pH of approximately 2 and 8, an adsorption edge exists, and virtually no metal adsorbs under pH 2 conditions. Therefore, instead of adjusting pH to 2 as some previous studies of desorption have done (e.g., Mamaril et al., 1997), a
more sensitive test of reversibility is to adjust the pH to a value on the adsorption edge. The pH of the suspension in each vessel was adjusted to each desired pH value with less than 100 μL of 2% HNO₃ or 0.5M NaOH. The pH of the reaction vessels was maintained at each step of the experiment through the use of an autotitrator in pH-stat mode. Samples were extracted from the reaction vessels, including a sample after the adsorption step but prior to desorption, and analyzed as described for the adsorption experiments.

2.2.4 Metal Desorption Experiments (pH dependence)

Batch desorption experiments were conducted as a function of pH, using polypropylene test tubes as reaction vessels. A known mass of washed bacteria was suspended in a known mass of 0.1M NaClO₄ electrolyte. A known mass of 1,000 ppm aqueous metal standard (Ca or Cd) was then added to the bacteria-electrolyte solution to create a homogeneous parent solution. The parent solution was adjusted to pH 8, and allowed to equilibrate for 1 hour. Aliquots of the parent solution were transferred to the reaction vessels, and the pH of the suspension in each vessel was adjusted to the desired lower pH values using small volumes of 2% HNO₃. The reaction vessels were placed on a rotating rack that provided gentle (20 rpm) end-over-end agitation for 2 hours. Metal desorption kinetics experiments in this study demonstrated that 2 hours is sufficient for desorption equilibrium to occur. The experimental suspension was then filtered through a 0.45 μm nylon filter. Each filtrate was acidified and analyzed for aqueous metal content by AAS.
2.3. Experimental Results

2.3.1. Cadmium Desorption Experiments

The observed Cd adsorption and desorption behaviors are depicted in Figure 2.1. Fein et al. (1997) determined that the carboxyl, phosphato, and hydroxyl sites on the cell wall of B. subtilis sequentially deprotonate with increasing pH, with pKa values of 4.8, 6.9, and 9.4, respectively. Therefore, at the pH of the adsorption and desorption experiments (pH 4), a significant fraction of the carboxyl sites are deprotonated, and hence negatively charged. Positively charged Cd$^{2+}$ is the dominant aqueous Cd species under these conditions, and it adsorbs onto the cell wall rapidly, reaching a steady-state extent of adsorption of 34 % within 2 hours. That is, we measured a 34% decrease in the aqueous concentration of Cd over the course of the experiment. Adsorption remains constant with a variation of $\pm 4\%$ (2$\sigma$) over the next 22 hours. The desorption process is even more rapid, attaining a steady-state value of 36% within 1 hour with a $\pm 6\%$ (2$\sigma$) change over the next 23.5 hours. For both the adsorption and desorption experiments, the change over 24 hours was not systematic in either direction. The effect of an extended adsorption contact time (17 hours compared to 1 hour) on the extent and kinetics of Cd desorption is depicted in Figure 2.2 (which also shows the Cd adsorption data from Figure 2.1). Desorption of the Cd is again rapid, and reaches a steady-state value of 28%. The extent of Cd adsorbed
Figure 2.1.

The percent of total Cd associated with the bacterial surface after adsorption (triangles) and desorption (circles) (after 1 hour of adsorption), as a function of time in 0.1M NaClO₄, with a total system concentration of Cd of 10⁻⁴M and a bacterial concentration of 10 g/L.
over the following 22 hours remains constant, with ± 5% (2σ), non-systematic variability. To determine if the adsorption maxima for Cd was stable for longer time periods, we performed a long term adsorption study. Figure 2.3 depicts the results of this experiment and demonstrates that the mass of Cd associated with the cell wall remains constant over a 3 day period. This verifies that there is no further adsorption or absorption of Cd associated with extended exposure of the bacteria to Cd.

The desorption behavior of Cd from the surface of B. subtilis as a function of pH is shown in Figure 2.4. The extent of desorption is negligible at high pH. That is, after the adsorption step at pH 8, only small quantities of Cd desorb into the experimental solutions whose pH was adjusted downward only slightly. With increasingly large adjustments toward lower pH values, the extent of desorption increases dramatically, until nearly all of the Cd desorbs into the solution adjusted to pH 2.5. This desorption edge is similar to the adsorption edge observed by Fein et al. (1997) under similar experimental conditions.
Figure 2.2.

The percent of total Cd associated with the bacterial surface after adsorption (triangles) and desorption (circles) (after 17 hours of adsorption), as a function of time in 0.1M NaClO₄, with a total system concentration of Cd of $10^{-4} \text{m}$ and a bacterial concentration of 10 g/L.
Figure 2.3.

The percent of total Cd associated with the bacterial surface after adsorption as a function of time in 0.1M NaClO₄, with a total system concentration of Cd of 10⁻⁴ M and a bacterial concentration of 10 g/L.
Figure 2.4.

The percent of total Cd associated with the bacterial surface after desorption (circles), as a function of pH in 0.1M NaClO$_4$, with a total system concentration of Cd of $10^{-4}m$ and a bacterial concentration of 10 g/L. Desorption began after a 1 hour period of adsorption at pH 8.2. pH was then lowered to between 2.5 and 7.5. The solid curve depicts the independent model generated by FITEQL (Westall, 1982) based on total metal and bacteria concentrations.
2.3.2. Calcium Desorption Experiments

A steady-state of 13% of the total Ca in the system adsorbed onto the bacterial surface within 30 minutes of initial contact with *B. subtilis* (Figure 2.5). As the experiment progressed, adsorption remained constant at $13 \pm 4\% \ (2\sigma)$ for nearly 25 hours, with no systematic changes as a function of time. Ca desorbed rapidly, attaining a steady-state within one hour at 12%. The mass of Ca adsorbed to the surface remained constant remained at $12 \pm 4\% \ (2\sigma)$, without any systematic behavior as the experiment progressed to 24 hours. Similar to the Cd experiments, the Ca adsorption and desorption kinetics experiments were conducted at pH 4.0. This pH is on the adsorption edge for Ca adsorption, and therefore represents a sensitive test of the extent of reversibility of the system.

Figure 2.6 shows the desorption kinetics of Ca from *B. subtilis* after 15 hours of adsorption at pH 8. Ca desorbs rapidly reaching a equilibrium at $11 \pm 5\% \ (2\sigma)$ adsorption within 1 hour. Similar to previous desorption experiments, the steady-state mass of metal on the surface of the bacteria is in good agreement with the adsorption data and displays no systematic behavior.

The desorption of Ca from *B. subtilis* as a function of pH is depicted in Figure 2.7. With decreasing pH, the mass of Ca on the cell wall of the bacteria decreases from a maximum amount of 25% of the total Ca in the system at pH 7.6, to 15% at pH 4.5, to nearly 0% adsorption at pH 2. Note that the maximum concentration of Ca
Figure 2.5.

The percent of total Ca associated with the bacterial surface after adsorption (triangles) and desorption (circles) (after 1 hour of adsorption), as a function of time in 0.1M NaClO₄, with a total system concentration of Ca of 10⁻⁴M and a bacterial concentration of 10 g/L.
Figure 2.6.

The percent of total Ca associated with the bacterial surface after adsorption (triangles) and desorption (circles) (after 15 hours of adsorption), as a function of time in 0.1M NaClO₄, with a total system concentration of Ca of 10⁻⁴M and a bacterial concentration of 10 g/L. Desorption began after a 15 hour period of adsorption at pH 8.6. pH was then lowered to 4.0 and maintained by an autotitrator.
Figure 2.7.

The percent of total Ca associated with the bacterial surface after desorption (circles), as a function of pH in 0.1M NaClO$_4$, with a total system concentration of Ca of $10^{-4}$M and a bacterial concentration of 6 g/L. Desorption began after a 1 hour period of adsorption at pH 7.6. pH was then lowered to between 2.5 and 7.0. The solid curve depicts the independent model generated by FITEQL based on total metal and bacteria concentrations.
on the bacterial surface under the higher pH conditions is significantly less than that observed in the Cd experiments. It is important to emphasize that the concentrations of Cd and Ca in each experiment are identical (\(10^{-4}\) molal), so our experiments (like those of Fowle and Fein, 1999) demonstrate that Ca exhibits a significantly lower tendency to be sequestered by the bacterial surface than does Cd. In addition, the desorption edge is similar to the Ca adsorption edge measured by Fowle and Fein (1999).

2.4. Discussion

2.4.1 Adsorption/Desorption Kinetics

In our experiments, both Cd and Ca adsorbed onto, and desorbed from, the bacterial surface rapidly, and after the initial reaction time, the concentration of metal bound to the bacterial surface remained constant for the duration of the experiments. Furthermore, regardless of whether the steady-state condition was approached from undersaturation (no metal initially associated with the bacterial surface) or from supersaturation (nearly all of the aqueous metal initially associated with the bacterial surface), the final concentration of metal bound to the bacterial surface was the same. Considered together, this evidence demonstrates that an equilibrium state exists controlling the concentration of metal associated with the bacterial surface relative to that free in solution. Our experiments demonstrate that chemical equilibrium modeling can be applied to quantify metal-bacteria adsorption/desorption reactions.
As stated above, after the initial adsorption or desorption, no further net reaction is observed for at least 24 hours (and up to 80 hours for Cd). Furthermore, the length of adsorption contact time exhibited no effect on the kinetics of desorption or on the concentration of metal associated with the bacterial surface following desorption. This suggests that the sorption sites are located at, or very close to, the bacterial surface, and that uptake of the metal into the interior of the cell is negligible under these non-metabolic experimental conditions. These results contradict those of Harvey and Leckie (1985) who described a two step uptake process associated with Pb binding to gram negative bacterium. In addition, Harvey and Leckie (1985) described a contact time dependence on the extent and kinetics of Pb adsorption and desorption. However it is not clear whether the discrepancies between our study and that of Harvey and Leckie (1985) arise because structural differences in the cell walls of gram negative and gram positive bacteria or the metabolic states of the bacteria in the studies.

2.4.2. pH Dependence

We test reversibility and the ability of surface complexation models to account for adsorption/desorption by comparing the observed pH dependent desorption behavior to that estimated using a chemical equilibrium surface complexation model (Figures 2.4 and 2.7, solid curves). The equilibrium constants used for chemical equilibrium modeling were determined in metal adsorption studies by Fein et al. (1997), and Fowle and Fein (1999). If the calculations (based on adsorption experiments) match the observations (of desorption behavior), then the agreement is
strong evidence for the existence of an equilibrium state. Calculation of the equilibrium state for each chemical species in the experiments is achieved by solving the set of mass balance and mass action constraints that define each system (Ca or Cd). Mass balance constraints are applied to the system in terms of total metal concentrations and total bacterial surface functional group site concentrations. These terms relate the known total concentrations to the sum of the concentrations of each species that contributes to the total concentration. Mass action equations relate species activities to equilibrium constants for each of the important reactions in the experimental systems. These reactions, the number of which depends on the composition of the experiment in question, include the bacterial surface deprotonation reactions for the carboxyl and phosphate functional groups:

\[(2.1) \quad R\text{-COO-H}^0 \leftrightarrow R\text{-COO}^- + H^+ \quad \text{pKa} = 4.82\]

\[(2.2) \quad R\text{-POO-H}^0 \leftrightarrow R\text{-POO}^- + H^+ \quad \text{pKa} = 6.9\]

as well as cation adsorption reactions involving aqueous divalent metal cations and each bacterial surface functional group:

\[(2.3) \quad R\text{-COO}^- + M^{2+} \leftrightarrow R\text{-COO-M}^+ \quad \log K \ (\text{Cd 3.4: Ca 2.7})\]

\[(2.4) \quad R\text{-POO}^- + M^{2+} \leftrightarrow R\text{-POO-M}^+ \quad \log K \ (\text{Cd 5.4})\]

where R represents the bacterium to which each functional groups is attached. We use equilibrium constants previously determined through acid-base titrations and batch adsorption experiments to constrain reactions (2.1)-(2.4) (Fein et al., 1997; Fowle and Fein, 1999). Using these equilibrium constants, with those of Wottery (1992) for aqueous reactions in the Na-CIO₄-NO₃ system, and with those reported by Baes and Mesmer (1976) for aqueous metal hydrolysis reactions, we can calculate the extent of
desorption expected under the experimental conditions. As with our previous models of metal adsorption onto bacterial surfaces, we use a constant capacitance model to account for the bacterial surface electric field effects on desorption, using FITEQL (Westall, 1982) to calculate these effects.

Figures 2.4 and 2.7 show the predicted percentage of total Cd and Ca, respectively, associated with the *B. subtilis* surface as a function of pH. The predicted extent of Cd adsorption (Figure 2.4) ranges from minimal adsorption at low pH (3.0 and below), to over 90% of the total Cd in the system at pH values of 6.0 and higher. For the Ca system, at pH values below 4.0, no Ca adsorption is predicted. With increasing pH, the estimated extent of adsorption increases to a maximum of 20% at pH 8.0. The observed amount of Cd and Ca associated with the cell wall after desorption is in very good agreement with these thermodynamic models. The Ca data show excellent agreement with the calculated model, while the Cd model exhibits only slight discrepancies with the data between pH 3 and 4. However, these discrepancies are within the expected uncertainty associated with the equilibrium constants previously determined for the Cd-*B. subtilis* surface complexes (Fein et al., 1997). That is, if we use the desorption data from this study to calculate stability constants for equilibria (2.3) and (2.4), for Cd or for Ca, we obtain values that are within the uncertainties of the stability constants derived from the adsorption studies by Fein et al. (1997) for Cd and by Fowle and Fein (1999) for Ca. Therefore, within the uncertainty of the experiments, the data provide conclusive evidence that the initial adsorption of these groundwater constituents onto *B. subtilis* is fully reversible.
2.5. Conclusions

The experimental results from this study demonstrate that the adsorption of Ca and Cd onto *Bacillus subtilis* is a rapid and completely reversible process. Furthermore, the results of the pH dependent desorption study are in excellent agreement with estimates of the amount of metal associated with the cell wall surface. These estimates are made using a surface complexation model with metal-bacteria stability constants derived from independent adsorption experiments. Therefore, the agreement between the desorption experiments and the estimates not only provides further evidence for the full reversibility of the adsorption/desorption equilibrium, but it also affirms the ability of surface complexation modeling to quantify metal bacteria adsorption/desorption reactions.

The results also suggest that the process of adsorption can be non-metabolic, and is dependent solely on the electrostatic and chemical attraction between the metal and the organic functional groups present on the cell wall of the bacteria. Metal adsorption does not appear to be specific to the cation in question, and transport of the metals into the cell cytoplasm does not appear to be occurring. If the metals were transported or diffused into the cell wall or cell interior, then we would expect to see hysteresis either in the extent or the kinetics of desorption. It is conceivable that minute quantities of metal pass into the cytoplasm over longer time periods than studied in our experiments, but our experimental results, combined with the electron microscopy study of Mullen et al. (1989), indicate that the vast majority of adsorbing
metal remains associated with the cell wall of the bacteria. Because metals are
attracted to the surface of bacteria, bacterial adsorption can significantly affect the
distributions, and hence mobilities, of both trace and major cations in groundwater
systems. Our results demonstrate that surface complexation modeling can successfully
be applied to quantify the speciation of metals in bacteria-water-rock systems.
References


CHAPTER 3

COMPETITIVE ADSORPTION OF METAL CATIONS ONTO TWO GRAM-POSITIVE BACTERIA: TESTING THE CHEMICAL EQUILIBRIUM MODEL

3.1. Introduction

Bacteria reside in many geological settings, from soil systems and groundwater aquifers, to hydrothermal vents and deep sedimentary basins (Kerr, 1997; Pennisi, 1997; McLean et al., 1996). In many of these systems, the cell walls of bacteria can represent a large percentage of the total surface area exposed to fluid. Bacterial cell wall surfaces exhibit a strong tendency to adsorb aqueous metal cations (e.g., Beveridge and Murray, 1976; 1980; Beveridge et al., 1982; Wood and Wang, 1983; Gonclaves et al., 1987; Xue et al., 1988; Konhauser et al., 1993). Therefore, bacteria have the potential to control major and trace metal geochemistry in water-rock systems through adsorption reactions (Beveridge and Murray, 1980; Mullen et al., 1989; McLean and Beveridge, 1990).
Natural systems, whether contaminated or not, typically contain numerous aqueous metal ions and a variety of mineralogical and biological surfaces. However, most previous studies of metal adsorption involving minerals or bacteria have examined the interactions that occur between a single metal and a single surface. Surface complexation chemical equilibrium theory offers a means to use experimental results from separate, isolated simple systems to estimate the distribution of mass (both dissolved and adsorbed) in more complex, multi-component systems. That is, experimentalists typically a specific metal-surface reaction is isolated and the equilibrium constant for that reaction is determined experimentally. The underlying assumption is that by knowing the equilibrium constants for each important reaction in a complex multi-component system, mass action and mass balance equations can be used to calculate the equilibrium state (the absolute concentrations of aqueous and surface species) of a system. The power of such an approach is that the complex system of interest does not need to be studied in the laboratory directly; only its component reactions need to be quantified. Geochemical equilibrium modeling enables recombination of these basic component reactions so that the equilibrium state can be determined for any system of interest. However, the accuracy of these calculated equilibrium states is not always checked through direct comparison with studied systems.

Some comparisons have been made between calculated and observed equilibrium states for multiple metal and/or multiple mineral surface systems (Benjamin and Leckie, 1981; Catts and Langmuir, 1985), but none have been undertaken for bacterial surfaces. Catts and Langmuir (1986) demonstrated that
equilibrium constants for individual metal-surface reactions that were determined from isolated single metal systems could be used to model the adsorption of Cu, Pb, and Zn onto a δ-MnO₂ mineral surface as a function of pH. Allan and Jarrell (1988) demonstrated that the constant capacitance model was effective in modeling the competition between protons and Cu for the sites of adsorption on maize and soybean root cell walls. Studies by Manning and Goldberg (1996) and Rybicka et al. (1995) have shown that chemical equilibrium models can successfully predict the adsorption of anions or cations onto competing clay minerals. However, to date no study has demonstrated the suitability of chemical equilibrium models to predict the distribution of adsorbing species onto competing bacterial surfaces.

Although some studies have examined competitive adsorption of metal cations onto bacterial cell wall surfaces, most experimental results cannot be extrapolated to systems that differ from those studied in the laboratory. For example, studies by Beveridge and Murray (1976), Mullen et al. (1989), and Mayers and Beveridge (1989) have quantified the ability of Bacillus subtilis to bind single or multiple metal cations through the use of system specific binding constants or Langmuir modeling. Cotoras et al. (1992) showed that the presence of competing cations inhibits the adsorption of uranium onto the surfaces of B. subtilis and Micrococcus. In each of these studies, however, a site-specific surface complexation approach was not invoked, and therefore the experimental results cannot be used to estimate the effect that competing cations would create in systems that differ from those studied.

Site-specific surface complexation modeling is not the only means of interpreting metal adsorption data, but it is the only approach that can account for
competitive adsorption effects as well as enabling extrapolation of experimental results to other systems of interest. Most experimental data from single metal-bacteria adsorption studies have been modeled using a distribution coefficient, $K_D$, and the related Freundlich isotherm (Mullen et al., 1989; Flemming et al., 1990). This approach, which quantifies $K_D$ as follows:

\[
K_D = \frac{[\text{Metal adsorbed}]}{[\text{Metal in solution}]}
\]

(3.1)

(where brackets denote concentration) assumes that an equilibrium exists according to the following ‘reaction’:

\[
\text{Metal in solution} \leftrightarrow \text{Metal adsorbed}.
\]

(3.2)

Whereas such an equilibrium between aqueous and adsorbed metal may exist, reaction (3.2) is an empirical representation of it. It does not depict the true balanced chemical reaction involving the sorbing surface. Equation (3.1) is not a mass action equation, and cannot be combined with other similar equations derived from simplified systems to quantify adsorption in multicomponent systems. Therefore, all studies based on these types of models are empirical and system specific, and are unable to represent competitive adsorption effects. Alternatively, the Langmuir isotherm approach differs from the Freundlich approach in that it explicitly accounts for surface equilibria (Stumm and Morgan, 1996; Langmuir, 1997). Furthermore, the Langmuir approach can be extended by additional parameters to describe competitive metal adsorption, but is also system specific because it does not explicitly account for the speciation of the adsorbing surface. If the surface speciation changes as a function of pH, ionic strength, or solution composition, then Langmuir modeling cannot be
used to estimate the extent of adsorption in systems not directly studied in the laboratory (Davis and Kent, 1990).

A non-ideal competitive adsorption (NICA) model (Koopal et al., 1994; Benedetti et al., 1995), developed to describe proton and metal adsorption onto humic and fulvic acids, has recently been applied to bacteria-metal reactions, specifically the binding of Ca$^{2+}$, Zn$^{2+}$, Cd$^{2+}$, and H$^+$ to *Rhodococcus erythropolis* A177 (Plette et al., 1996). The NICA model invokes affinity distribution functions to describe the heterogeneity of proton and cation binding to the bacterial surface. In effect, the approach adjusts Langmuir-Freundlich (ideal) adsorption models to account for sorbate interactions with specific surface functional groups. Although the complexity of the NICA model appears to be necessary for quantifying proton and metal adsorption onto humic and fulvic acids (Koopal et al., 1994; Benedetti et al., 1995), bacterial surface reactions can be successfully modeled more simply with discrete equilibrium constants rather than the affinity distribution functions of the NICA model (Fein et al., 1997; Daughney et al., 1998; Daughney and Fein, 1998). This suggests that the heterogeneity of the adsorption sites in the repeating polymers in bacterial cell walls are not as pronounced as those found in humic and fulvic acids.

The objective of this study is to test whether a discrete equilibrium constant surface complexation model can successfully account for the bacterial adsorption of aqueous metal cations based solely on independently determined stability constants for metal-bacteria surface complexes. Specifically, we measure competitive adsorption in either multi-metal single bacteria systems, single metal systems containing two species of bacteria, or multi-metal systems containing two species of
bacteria. We test the ability of the chemical equilibrium approach to account for metal distributions in these systems by comparing the measured extents of adsorption to those calculated using independently determined equilibrium constant values from single metal experiments. The results of Fowle and Fein (1999) demonstrate that the adsorption of Cd and Ca is a fully reversible process. No hysteresis between the extent or the kinetics of desorption and adsorption was observed, indicating that irreversible transport of metals into the cell wall or cytoplasm does not occur to a significant extent. Although it is possible that small quantities of metal pass into the cell interior, the results of Fowle and Fein (1999) coupled with the electron microscopy results of Mullen et al. (1989) indicate that the vast majority of the adsorbing metal is bound to the cell wall of the bacteria. Our previous studies (Fein et al., 1997; Daughney et al., 1998; Daughney and Fein, 1998) demonstrate that a discrete equilibrium constant model can successfully account for adsorption behavior in a single metal-single bacterial species system. This study determines whether a discrete equilibrium constant model can be extended to systems that contain numerous aqueous metals and sorbing bacterial surfaces.

3.2. Experimental Procedure

3.2.1. Bacteria Growth Procedures

The bacterial species *B. subtilis* and *B. licheniformis* were prepared and cultured following the procedure outlined in Fein et al. (1997), and Daughney et al. (1998), except that the bacteria were washed in 1 mM EDTA for only 1 hour, and the electrolyte used was 0.1M NaClO₄. All solutions in this study were prepared with 18
MΩ water. The wash protocol ensures that the cell walls of the bacteria are stripped of competing metals. Without a thorough wash, the total concentration of metals in the system would be unknown, and the equilibrium states could not be calculated because the mass balance constraints would be unknown. Prior to each experiment, the bacteria were pelleted by centrifugation at 7500 rpm for 60 min. The mass of the pellet was measured in order to determine the concentration of surface functional groups in each experiment. Note that the weight of bacteria used in each experiment is not reported as a dried weight, but as a weight after centrifugation.

3.2.2. Metal Adsorption Experiments

Batch adsorption experiments were conducted as a function of pH, using polypropylene test tubes as reaction vessels. Washed bacteria were suspended in 0.1M NaClO₄ electrolyte, and 1000 ppm aqueous metal standard (Ca, Cu, Cd, and/or Pb) was added to the bacteria-electrolyte solution to create a homogeneous parent solution of known bacterial and metal concentrations. Aliquots of the parent solution were transferred to the reaction vessels, and the pH of the suspension in each vessel was adjusted to the desired pH value using small volumes (less than 1% of the total volume) of 10% HNO₃ or 0.5M NaOH. The reaction vessels were placed on a rotating rack that provided gentle (20 rpm) end-over-end agitation for 1 hour. Metal adsorption kinetics experiments by Fein et al. (1997) demonstrated that 1 hour is sufficient for adsorption equilibrium to occur. The reaction mixture was then filtered through a 0.45 μm nylon filter. Each filtrate was acidified and analyzed for aqueous metal content by inductively coupled plasma mass spectrometry (ICP-MS). The types
of experiments that we conducted (listed in detail in Table 1.1) are as follows: 1) single metal, single bacteria; 2) single metal, two species of bacteria; 3) multiple metal, single species of bacteria; and 4) multiple metals, 2 species of bacteria. Batch control experiments (Cu, Cd, Pb mixed metal) were conducted as function of pH to determine whether precipitation or metal loss to the reaction vessels occurred. Control experiments demonstrated that significant precipitation of Cu occurred at pH values above 6.5 in solutions containing the same Cu concentrations as those used in our experiments. Our experimental approach measures bulk metal removal from solution, and cannot distinguish between adsorption and precipitation. Therefore, we only illustrate and model the Cu measurements for experimental pH values below 6.5. The single metal, single bacteria experiments were designed to explore for possible metal-bacteria surface species not identified by previous investigations.

The adsorption behaviors of Pb and Cu ions onto the surface functional groups of B. subtilis and B. licheniformis are defined by Fein et al. (1997) and Daughney et al. (1998). However, due to the pH ranges investigated, and the surface:solute ratios used in their experiments, Fein et al. (1997) and Daughney et al. (1998) constrain only divalent aqueous cation adsorption. Their data do not require the adsorption of cation hydrolysis products or electrolyte complexes. To probe for the existence of such surface complexes we have conducted a series of experiments at low bacteria:metal ratios, for Cu and Pb. To model competition between major and trace cations, we have quantified Ca adsorption onto B. subtilis using two sets of single metal adsorption experiments conducted at different bacteria:Ca ratios.
3.2.3. Competitive Bacterial Surface Experiments

Pellets of *B. subtilis* and *B. licheniformis* were weighed and suspended together in 0.1M NaClO₄, and 1000 ppm aqueous metal standard (Cd alone, or in combination with Cu and Pb) was added to the mixture to create a homogenous parent solution. The single metal adsorption procedure was then repeated. See Table 3.1 for exact experimental concentrations.

3.2.4. ICP-MS Analyses

The ICP-MS analyses were conducted using a Fisons Instruments VG PlasmaQuad model PQII STE. The machine was operated in peak jumping mode, and displayed a stability of < 2% across the mass range of interest in all analyses. Samples were blank subtracted by using high purity, double-distilled 1% HNO₃ as a blank, and internal standards which bracketed the masses of interest were used to correct for machine drift. Calibrations were carried out by using 5-8 standard solutions prepared from 1000 ppm standard stock solutions. All samples, blanks, and standards were prepared with double distilled reagents and 18 MΩ water.

Table 3.1 Metal adsorption experimental conditions

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<th>Series</th>
<th>Bacteria¹</th>
<th>log ( m_{\text{carboxyl}} )²</th>
<th>Metal</th>
<th>log ( m_{\text{initial}} )³</th>
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<tr>
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</tr>
</tbody>
</table>

¹ BS: *Bacillus subtilis*; BL: *Bacillus licheniformis* ² Concentration of carboxyl sites in moles/kg of water ³ Initial aqueous metal concentration
3.3. Experimental Results

3.3.1. Single Metal Adsorption Experiments

The observed single metal adsorption behaviors of Cu and Pb onto *B. subtilis* are depicted in Figures 3.1 and 3.2, respectively. At low pH values, the organic functional groups located in the cell wall of the bacteria are fully protonated, and therefore no adsorption of metal ions occurs. As pH increases, the surface functional groups of *B. subtilis* deprotonate successively, resulting in an increasing number of sites available for metal adsorption. The positively charged Cu and Pb ions are electrostatically attracted to the negatively charged sites, and adsorption occurs. Cu begins to adsorb at pH 3.5, with adsorption increasing with increasing pH until a maximum of approximately 70% adsorption is attained at pH 6.5. Pb adsorption is minimal, but measurable, at pH 2, and the extent of Pb adsorption increases with increasing pH to approximately pH 9. As pH increases above 9, Pb adsorption decreases slightly.

The adsorption of Ca onto the surface of *B. subtilis* (Figure 3.3) follows a similar trend to that exhibited by Cu and Pb. That is, under low pH conditions, adsorption is negligible, and with increasing pH, adsorption increases. However, the extent of Ca adsorption onto the bacterial surface is much less than that observed for Cu or Pb, with a maximum observed extent of adsorption at pH 8.
Figure 3.1.

Cu (1.57×10^{-4} \text{ m}) adsorption onto \textit{B. subtilis} (0.80 \text{ g/L}). Curves represent fits generated by FITEQL: dashed curve represents model with only Cu^{2+}-carboxyl and -phosphato surface complexation; solid curve represents best-fitting model with both Cu^{2+}- and CuOH^{+}-carboxyl surface species present.
Figure 3.2.

Pb (4.81x10^{-5} m) adsorption onto B. subtilis (0.80 g/L) as a function of pH. Dashed curve represents model with only Pb^{2+}-carboxyl and -phosphato surface complexation; solid curve represents model with both Pb^{2+} and PbOH^{+}-carboxyl surface species present.
3.3.2. Multiple Metal Adsorption Experiments

The competitive adsorption behavior of Cu, Pb, and Cd onto *B. subtilis* is depicted in Figures 3.4 and 3.5. These figures represent experiments conducted using the same concentrations of metals, but with different bacteria concentrations (6.58 g bacteria/L and 0.80 g bacteria/L, respectively). The general pH trend for each metal follows that of the individual metals, with little or no adsorption under low pH conditions, and increasing adsorption with increasing pH. Figures 3.4 and 3.5 both illustrate that Cu, Pb, and Cd each exhibit adsorption edge behavior, with the Cu edge occurring at the lowest pH values, followed by Pb and Cd. The adsorption edges shown in Figure 3.5 are not as steep as those in Figure 3.4 because the metal:bacteria ratio used is higher than that used for the experiments in Figure 3.4. Because less bacterial surface is available, less adsorption occurs at a given pH value, and not all of the aqueous metal adsorbs, even at the highest pH values studied.

Figures 3.6 and 3.7 depict results from experiments conducted under the same conditions as the experiments shown in Figures 3.4 and 3.5, but in these cases *B. licheniformis* was used in the experiments. Because the cell wall composition and acid/base behavior differs between the two bacterial species, the adsorption edges shown in Figures 3.6 and 3.7 are not replicas of those in Figures 3.4 and 3.5, despite using identical bacteria weights and metal concentrations in the experiments. However, the general pH trends, and adsorption edge sequences are similar. As is the case for *B. subtilis*, the experiments conducted with a bacteria concentration of 0.80
Figure 3.3.

Ca adsorption onto *B. subtilis* as a function of pH. The circles correspond to experiments with 6.58 g bacteria /L and $10^4$ m total Ca; The squares correspond to experiments with 6.03 g bacteria /L and $10^4$ m total Ca. The curves represent the model for each bacterial concentration, calculated using the best-fitting value of the equilibrium constant.
Figure 3.4.

Cu ($4.76 \times 10^{-5} \text{ m}$, circles), Pb ($1.47 \times 10^{-5} \text{ m}$, triangles), and Cd ($2.71 \times 10^{-5} \text{ m}$, squares) adsorption onto *B. subtilis* (6.58 g/L). Curves represent independent models generated by FITEQQL based on total metal and bacteria concentrations: solid line Cu; dashed line Pb; gray line Cd.
Figure 3.5.

Cu (4.71x10^{-5} m, circles), Pb (1.45x10^{-5} m, triangles), and Cd (2.67x10^{-5} m, squares) adsorption onto B. subtilis (0.80 g/L). The meaning of the curves is the same as in Figure 3.4.
g/L yield less adsorption of each metal at a given pH than do the experiments conducted at a concentration of 6.58 g/L. Because not all of the aqueous metal is adsorbed, even under the highest pH conditions of the experiments, the low ratio experiments provide a more sensitive test of the predictive capabilities of the modeling approach.

The effects of $10^{-3} \text{m}$ Ca on the adsorption of the trace metal Cd (at a concentration of $10^{-5} \text{m}$) are illustrated in Figure 3.8. The relative concentrations of the two cations are similar to that found in contaminated aquifers. Although Ca was present in great excess of Cd, its affinity for the bacterial functional groups was much lower than that of Cd, thereby enabling the trace metal to effectively compete for the cell wall sites. The general pH trends for Ca and Cd are similar to the individual metals, with little adsorption below pH 3.5, and adsorption maxima at pH 6 for Ca and pH 6.8 for Cd.

3.3.3. Multiple Bacteria Metal Adsorption Experiments

The adsorption behaviors in systems containing both bacterial species are illustrated in Figures 3.9 and 3.10. The first experiment included $10^{-4} \text{M}$ Cd and 3.2 g/L of each species of bacterium. Adsorption proceeded similarly to single bacteria experiments with no observable colloidal effects or flocculation between the particles. The Cd adsorption trend is similar to the results of single bacteria adsorption experiments of Fein et al. (1997) and Daughney et al. (1998), with little adsorption below pH 1.0 and the highest amount of adsorption at pH 8.0.
The competitive adsorption of Cu, Pb, and Cd onto both *B. subtilis* and *B. licheniformis* is shown in Figure 3.10. The figure depicts the overall extent of removal of each metal. The distribution of each metal between the two bacterial surfaces could not be determined with the experimental procedure. However, the overall shape and order of the adsorption edges are consistent with the previous experiments.

3.4. Equilibrium Modeling and Discussion

Calculation of the equilibrium state for each chemical species in the experiments is achieved by solving the set of mass action and mass balance constraints that define each system. The set of equations varies depending on the conditions of each experiment. Mass balance constraints are written for total metal concentrations and for total bacterial surface functional group site concentrations, relating the known total concentrations to the sum of the concentrations of each species that contributes to the total concentration. Mass action equations relate species concentrations to equilibrium constants for each of the important reactions in the experimental systems.

These reactions, the number of which also depends on the starting composition of the experiment in question, include the bacterial surface deprotonation reactions for the carboxyl, phosphate, and hydroxyl functional groups:

(3.3) \[ \text{R-COOH} \rightleftharpoons \text{R-COO}^- + \text{H}^+ \]

(3.4) \[ \text{R-POOH} \rightleftharpoons \text{R-POO}^- + \text{H}^+ \]

(3.5) \[ \text{R-OH} \rightleftharpoons \text{R-O}^- + \text{H}^+ \]
Figure 3.6.

Cu (4.68x10^-5 \text{ m}, circles), Pb (1.44x10^-5 \text{ m}, triangles), and Cd (2.64x10^-5 \text{ m}, squares) adsorption onto \textit{B. licheniformis} (6.58 \text{ g/L}). The meaning of the curves is the same as in Figure 3.4.
Figure 3.7.

Cu ($4.72 \times 10^{-5} \text{ m}$, circles), Pb ($1.45 \times 10^{-5} \text{ m}$, triangles), and Cd ($2.67 \times 10^{-5} \text{ m}$, squares) onto *B. licheniformis* (0.80 g/L). The meaning of the curves is the same as in Figure 3.4.
Figure 3.8.

Ca (9.26x10^{-4} m, circles) and Cd (9.53x10^{-6} m, squares) adsorption onto B. subtilis (4.63 g/L). Curves represent independent models generated by FITEQL based on total metal and bacteria concentrations: solid black line Ca; gray line Cd.
Figure 3.9.

Cd ($8.97 \times 10^{-5}$ m, squares) adsorption onto *B. subtilis* (3.26 g/L) and *B. licheniformis* (3.13 g/L). Curves represent independent models generated by FITEQL based on total metal and bacteria concentrations: solid black line Cd.
Cu (4.72x10^{-5} m, circles), Pb (1.45x10^{-5} m, triangles), and Cd (2.67x10^{-5} m, squares) adsorption onto *B. subtilis* (0.80 g/L) and *B. licheniformis* (0.80 g/L). Curves represent independent models generated by FITEQL based on total metal and bacteria concentrations: solid line Cu; dashed line Pb; gray line Cd.
as well as cation adsorption reactions involving aqueous divalent metal cations and each bacterial surface functional group:

\[
(3.6) \quad \text{R-COO}^- + M^{2+} \rightleftharpoons \text{R-COO-M}^+ \\
(3.7) \quad \text{R-POO}^- + M^{2+} \rightleftharpoons \text{R-POO-M}^+
\]

Where R, represents the bacterium to which each functional group is attached. We neglect metal adsorption reactions with surface hydroxyl sites, although these interactions may become important at pH values above approximately 9. The experiments described by Fein et al. (1997) and Daughney et al. (1998) did not cover as wide a pH range nor metal:bacteria mass ratio range as those reported in this study. Therefore, it is possible that additional bacterial surface species become important in our experiments. For example, both Cu and Pb form hydrolysis products within the pH range of the experiments, and it is likely that at least the positively charged first hydrolysis product adsorbs onto the bacterial surface (written here for the carboxyl site, but interaction with the phosphato site is possible as well):

\[
(3.8) \quad \text{R-COO}^- + \text{MOH}^+ \rightleftharpoons \text{R-COO-MOH}^0
\]

In addition to equilibria involving the bacterial surface functional groups, we account for aqueous complexation and water dissociation in the equilibrium modeling.

We can estimate the speciation and distribution of each metal in the experiments if equilibrium constants are known for the equilibria described above. Equilibrium constants for the surface deprotonation reactions (equilibria 3.3-3.5) and divalent metal adsorption reactions (equilibria 3.6 and 3.7) have been determined by Fein et al. (1997) for \textit{B. subtilis}, and by Daughney et al. (1998) for \textit{B. licheniformis},
respectively. We use our experimental results to quantify the Ca\textsuperscript{2+}-\textit{B. subtilis} (Figure 3.3) adsorption reaction, and we extend the experiments of Fein et al. (1997) to higher pH values to determine if Cu and Pb hydrolysis products adsorb onto the surface of \textit{B. subtilis}. With these equilibrium constants, and with those of Wolery (1992) for aqueous reactions in the Na-ClO\textsubscript{4}-NO\textsubscript{3}-OH-H\textsuperscript{+} system, and with those reported by Baes and Mesmer (1976) for aqueous metal hydrolysis reactions, we can calculate the extent of adsorption expected under the experimental conditions for the mixed metal and/or mixed bacterial systems. As with our previous models of metal adsorption onto bacterial surfaces, we use a constant capacitance model to account for the bacterial surface electric field effects on adsorption, using FITEQL (Westall, 1982) to calculate these effects.

3.4.1. Single Metal Adsorption Experiments

The estimated extent of adsorption, as a function of pH, is illustrated as dashed curves in Figures 3.1 and 3.2 for Cu and Pb, respectively. There is reasonable agreement between the estimated and observed adsorption behaviors from the lowest pH values examined to approximately pH 6. However, both the Cu and the Pb experiments document that adsorption continues to increase with increasing pH above pH 6. The models predict that the extent of adsorption should plateau and ultimately decrease due to the formation of aqueous hydrolysis complexes under high pH conditions. The misfit between the observations and the predictions increases with increasing pH, suggesting that the cause of the enhanced adsorption is the presence of a metal-bacteria surface species that was not accounted for in the equilibrium
modeling. We determined the stoichiometry of the metal-bacteria surface complex that best accounts for the observed misfit by testing a wide range of possible adsorbing species stoichiometries, including MOH$^+$, MOH$^0$, MClO$_4^+$, M$_2$(OH)$_2^{2+}$, and M$_2$(OH)$^{3+}$ (where M represents either Cu or Pb). In addition we tested the adsorption of each of these species onto both the surface carboxyl and phosphato sites. We also tested whether M$^{2+}$-hydroxyl surface complexation could account for the misfit. We used the variance function, V(Y), calculated by FITEQL, to determine the reaction stoichiometry that best fits the experimental data. Of all of the above possibilities, the lowest V(Y) values correspond to models that account for the enhanced adsorption effects with CuOH$^+$ and PbOH$^+$ adsorption onto surface carboxyl sites.

We use FITEQL with a constant capacitance model (Westall, 1982) to determine the equilibrium constants for the following adsorption reactions:

(3.9) \[ \text{CuOH}^+ + \text{R-COO}^- \Leftrightarrow \text{R-COO-CuOH}^0 \]

(3.10) \[ \text{PbOH}^+ + \text{R-COO}^- \Leftrightarrow \text{R-COO-PbOH}^0 \]

Aqueous mass balance calculations demonstrate that metal hydroxide complexes begin to dominate aqueous metal solution chemistry in the same pH range where enhanced adsorption is observed. Figure 3.1 illustrates that inclusion of the additional R-COO-CuOH$^0$ species in the equilibrium model (solid curve) significantly improves the fit to the experimental data, with excellent agreement now occurring throughout the pH range of the experiment. Figure 3.2 depicts the model predictions for Pb$^{2+}$ adsorption (dashed curve) as well as for Pb$^{2+}$ and PbOH$^+$ adsorption (solid curve). Both models slightly misfit the data at low pH values, and with increasing pH the
Pb\(^{2+}\)-only model significantly under-predicts the mass of metal adsorbing to the surface functional groups. However, the inclusion of the R-COO-PbOH\(^0\) surface species in the model dramatically improves the fit to the experimental data. The best-fitting stability constants calculated for each type of experiment, are compiled in Table 3.2. Similar experiments with the organism *B. licheniformis* were not conducted. However, predictions of the stability constant values were made by interpolation of a least squares correlation between the metal-bacteria stability constants for *B. subtilis* and *B. licheniformis* from Daughney et al. (1998), and the results of these calculations are also listed in Table 3.2.

**Table 3.2 Metal-bacteria stability constants**

<table>
<thead>
<tr>
<th>Metal Species</th>
<th>Bacteria(^1)</th>
<th>Adsorption Site</th>
<th>log K</th>
</tr>
</thead>
<tbody>
<tr>
<td>PbOH(^+)</td>
<td>BS</td>
<td>Carboxyl</td>
<td>5.8±0.1</td>
</tr>
<tr>
<td>CuOH(^+)</td>
<td>BS</td>
<td>Carboxyl</td>
<td>6.4±0.1</td>
</tr>
<tr>
<td>PbOH(^+)</td>
<td>BL</td>
<td>Carboxyl</td>
<td>6.6±0.8(^a)</td>
</tr>
<tr>
<td>CuOH(^+)</td>
<td>BL</td>
<td>Carboxyl</td>
<td>6.1±0.9(^a)</td>
</tr>
<tr>
<td>Ca(^{2+})</td>
<td>BS</td>
<td>Carboxyl</td>
<td>2.8±0.1</td>
</tr>
</tbody>
</table>

\(^1\) BS: *Bacillus subtilis*; BL: *Bacillus licheniformis*  
\(^a\) Estimated log K; Error based on regression analysis

With a full description of Cd, Cu, and Pb speciation on the bacterial surfaces, the last component required in order to predict the extent of adsorption that should occur in the mixed metal and/or mixed bacteria systems is the equilibrium constant values for Ca adsorption onto *B. subtilis*. Figure 3.3 depicts the model fits and experimental data for the adsorption of Ca onto the surface of *B. subtilis*. To determine the stoichiometry of the adsorbed Ca species on the surface of *B. subtilis*, a number of models were tested including: Ca\(^{2+}\), CaOH\(^+\), or CaHCO\(_3\)\(^+\) adsorbing onto
the carboxyl or phosphato sites; Ca$^{2+}$ binding to two carboxyl sites; and Ca$^{2+}$ adsorbing onto the carboxyl and phosphato sites. The experimental data were used to determine the best equilibrium constant and stoichiometry for the adsorbed Ca species. Comparisons of the FITEQL variance function, $V(Y)$, for each stoichiometry indicated that the best fitting model was the adsorption of Ca$^{2+}$ onto the surface carboxyl sites of *B. subtilis*:

\[(3.11) \quad \text{Ca}^{2+} + R\text{-COO}^- \rightleftharpoons R\text{-COO-Ca}^+\]

Figure 3.3 depicts the experimental data and model predications for the adsorption of Ca onto *B. subtilis* at two different bacteria concentrations. Each data set yields a best-fitting stability constant value for the surface R-COO-Ca$^+$ species, and the model predictions shown in Figure 3.3 calculated using the average stability constant value from the two data sets. Each curve is in excellent agreement with its corresponding data from pH 2-8. Above pH 8, the 6.58 g/L experimental data are slightly under-predicted.

3.4.2. Multiple Metal Adsorption Experiments

Our single metal, single bacteria adsorption experiments complete the set of equilibrium constant values that are required in order to predict the extent of adsorption that should occur in the multiple metal experiments. Comparing the estimated and observed simultaneous adsorption behaviors of three metals (Cu, Cd, and Pb) onto *B. subtilis* or *B. licheniformis* results in a powerful test of the application of the chemical equilibrium approach to bacterial surface complexation. The independent modeling of the competitive adsorption of Cu, Pb, and Cd onto *B.
*subtilis* at a high solid/solution ratio is depicted in Figure 3.4. The curves shown in the figure represent the total adsorption percentage, and account for all of the metal surface species. Each independently predicted curve is in excellent agreement with its corresponding data. The figure demonstrates that the chemical equilibrium approach provides an excellent fit to the observed competitive adsorption behavior of Cu, Pb, and Cd across the entire pH range studied. Figure 3.5 depicts the observed and modeled adsorption of the metals under conditions (at a lower solid:solution ratio) that promote the competition between the metals for bacterial surface adsorption sites. The model prediction of the Cu adsorption behavior is in excellent agreement with the experimental measurements of Cu adsorption throughout the pH range studied (< pH 6.5). The estimated extent of adsorption for Pb and Cd are in good agreement with the experimental results, but at above approximately pH 7.5, the model predicts Pb adsorption that is 6-10% higher than the observations.

Comparisons of the results from the multi-metal *B. licheniformis* experiments and the model predictions are shown in Figures 3.6 and 3.7. In general, the extents of adsorption and the pH dependence of adsorption are described very well by chemical equilibrium modeling of the competitive adsorption. Similar to the multi-metal, *B. subtilis* experiments, the independent modeling for Pb and Cu adsorption depicted in Figure 3.6 for the high solid:solution ratio experiments is in excellent agreement with the experimental results for each metal, across the entire pH range. However, from pH 3-6 the observed extents of Cd adsorption are 10-20% higher then those predicted by the model. For the experiments with a low solid:solution ratio, the extent of adsorption is predicted accurately for each metal at all pH values below
approximately 6.0. Similar to the results from the high solid/solution ratio experiments, above pH 6.0 the model under-predicts the extent of Cd adsorption by 10-15%. This misfit is most likely due to inaccuracies in the Cd-phosphato surface complex stability constants determined by Daughney et al. (1998). The experiments conducted by Daughney et al. (1998) provided tight constraints on the metal-carboxyl surface complex stability constants, but due to the metal:bacteria ratios used by Daughney et al. (1998), the stability constants derived for metal-phosphato surface complexes are more uncertain. These uncertainties do not significantly affect the speciation calculation for the higher solid:solution ratio (shown in Figure 3.6) because all of the aqueous metal is adsorbed onto carboxyl sites, with little or none left in solution under higher pH conditions to interact with phosphato sites. We use our high solid:solution ratio data to place constraints on the values of the Cd-phosphato surface complex stability constants. The best-fitting values for the log K for the Cd-surface complex is 4.2, and this value is within the uncertainty associated with the value reported by Daughney et al. (1998). This example does not negate the effectiveness of the chemical equilibrium approach, but rather it underscores the importance of obtaining accurate stability constant values in order to produce accurate models of the effects of bacteria on metal adsorption behavior.

Figure 3.8 illustrates the estimated and observed extents of competitive adsorption of two cations onto the surface of B. subtilis. These experiments were designed to test the ability of the chemical equilibrium model to account for competitive adsorption of a trace element (Cd) in the presence of a major groundwater constituent (Ca). The agreement between the data and the theoretical prediction is
excellent, except for a slight under-prediction of the extent of adsorption by the model at the lower pH values of the experiments. Even though Ca concentrations were more than two orders of magnitude higher than those of Cd in the system, higher thermodynamic stabilities of the Cd-bacterial surface complexes compared to those involving Ca leads to nearly complete adsorption of the aqueous Cd under mid- to high-pH conditions. This experiment underscores the electrostatic nature of the adsorption reactions. Although Ca is an important element for cell metabolism and structure and Cd is toxic to the cell, the adsorption behaviors of these two elements are not reflections of the different effects they have on the cell. Metals are not selectively bound to the bacteria cell wall surface via any metal-specific interactions that are designed to attract beneficial cations to the cell. Rather, they are attracted predominantly according to their electrostatic properties. Because these properties manifest themselves in terms of the value of the metal-bacterial site stability constants, chemical equilibrium modeling provides a means for accounting for metal distributions in bacteria-bearing systems as long as these stability constants have been measured or estimated. Because many trace metal contaminants interact strongly with carboxyl and phosphato sites, while many major groundwater constituents (such as Ca, Na, and Mg) do not, trace metals compete effectively with major elements for the available sites, and bacteria can significantly affect the distribution of both trace and major elements.
3.4.3. Multiple Bacteria Adsorption Experiments

The measured and predicted adsorption behaviors for the systems that contained both types of bacteria simultaneously are depicted in Figures 3.9 and 3.10. Because FITEQL 2.0 cannot account for the electric field effects arising from two surfaces simultaneously, we modeled the electric potential effects of the bacterial surfaces by considering the surface functional group concentrations to be independent from one another, but for each surface to exhibit a common 'averaged' electric potential. That is, we invoked a constant capacitance model that used a capacitance value (5.5 C/m²) that is the average between the *B. subtilis* and the *B. licheniformis* capacitance values. Figure 3.9 shows excellent agreement between the independent modeling and the experimental results for the adsorption of Cd onto the two bacteria throughout the entire pH range. Figure 3.10 depicts the adsorption of Cu, Cd, and Pb onto the two bacteria. The agreement between the experimental measurements for Cu, Pb, and Cd adsorption, and the theoretical model is again excellent.

3.5. Conclusions

In this study, we demonstrate that the use of a chemical equilibrium model of metal adsorption onto bacterial surfaces can accurately predict the distribution of metals in complex systems. In chemical equilibrium modeling, aqueous and surface speciation can be calculated if equilibrium constants for the component reactions are known. We use previously determined Cd²⁺-, Cu²⁺-, and Pb²⁺-carboxyl and phosphate stability constants, along with stability constants for the Ca²⁺-, CuOH⁺-,
and PbOH⁻-carboxyl surface species that are determined in this study, to calculate the equilibrium distribution of species in mixed metal, mixed bacteria systems. The extent of metal adsorption in each multi-component system is estimated independently from the experimental measurements, so the comparison constitutes a rigorous test of the estimation procedure. We tested the equilibrium approach on three types of multi-component systems: 1) systems containing one metal and both B. subtilis and B. licheniformis; 2) those containing several metals and one of the bacterial species studied; and 3) those containing several metals and both bacterial species. The experiments were conducted as a function of both pH and metal:bacteria concentration ratio. Although some of the systems studied exhibited differences between the observed and estimated extents of adsorption, in all cases these differences were slight and within the uncertainties of the estimation procedure. The chemical equilibrium calculations successfully accounted for the competition between adsorbing cations, for the competition between adsorbing surfaces, for the pH dependence of adsorption, and for the extent of adsorption as a function of metal:bacteria ratio. These results are a strong affirmation that the chemical equilibrium approach represents an accurate method for calculating the effects of bacteria on metal adsorption in multi-component systems not directly studied in the laboratory.

The experimental results also emphasize that metal adsorption onto bacterial surfaces is a metabolically independent process. Whether they are inorganic nutrients for bacterial cells (such as Ca) or are potentially toxic to the cells (such as Cd, Cu, and Pb), aqueous metals adsorb according to their electrostatic properties. Although the
mechanisms for transport of metals through the cell wall and membrane structures may be tailored to specific metal cations, our results indicate that the cell wall does not exhibit 'preferential' or metal-specific adsorption properties. Because, in general, trace metal cations exhibit higher affinities for bacterial surface functional groups than do major element cations, trace metals can effectively compete with the much more abundant aqueous cations, such as Ca$^{2+}$, for the available bacterial surface sites. Therefore, bacterial adsorption can significantly affect the distributions, and hence mobilities, of both trace and major cations in groundwater systems.
References


CHAPTER 4

EXPERIMENTAL STUDY OF URANYL ADSORPTION ONTO BACILLUS SUBTILIS

4.1. Introduction

The release and subsequent transport of uranium and other radionuclides in near-surface geological systems has focused research on developing accurate and versatile predictive tools for determining the fate of uranium in water-rock systems. The mobility of uranium in low temperature water-rock systems is controlled by the solubility of secondary uranium mineral phases (Burns et al., 1997; Murakami et al., 1997), the sorption of uranium to inorganic and biological substrates (Waite et al., 1994; Turner et al., 1996; Suzuki and Banfield, 1999), the redox chemistry of uranium (Grenthe, 1992; Lovely et al., 1991; Lovely and Phillips, 1992; Lovely et al., 1993), and the tendency of uranium to form stable aqueous complexes (Grenthe, 1992). Significant efforts have been made to develop a thermodynamic database that can accurately describe the aqueous geochemistry of uranium in these systems (Grenthe, 1992; Shock et al., 1997; Langmuir, 1997). However, without a means to
quantify the interactions between microorganisms and uranium (and other radionuclides), it remains difficult to incorporate uranium-bacteria interactions into current contaminant transport models.

Bacterial sorption may affect the fate of uranium in many near-surface environments. Laboratory and field studies have demonstrated that microbes have the ability to facilitate the removal of uranium from the aqueous phase through the sorption of U(VI) to bacterial cell walls (Haas et al., 1998; Suzuki and Banfield, 1999), through the biological reduction of U(VI) (Lovely et al., 1991; Lovely and Phillips, 1992; Lovely et al., 1993), and through enzymatic production or nucleation of U mineral precipitates (Mackaskie et al., 1992; Mann and Fyfe, 1985). The high affinity of bacteria for U and other radionuclides has led to a number of studies which measure the binding capacity of a particular microorganism under a unique set of experimental conditions (for a thorough review of these studies, see Suzuki and Banfield, 1999). Many researchers have investigated uranium sorption onto microbial cell wall surfaces. For example, studies by Friis and Myers-Keith, (1985) and Cotoras et al. (1992) quantified the ability of *Streptomyces longwoodensis* and *Micrococcus* to bind U through the use of system specific binding constants or chemical equilibria modeling. In each of these studies, however, a site-specific surface complexation approach was not invoked, and therefore the experimental results cannot be used to estimate the effect that changes in pH, or other aqueous chemistry would have on adsorption. Each of these studies has helped to demonstrate that bacteria are likely to play a significant role in the transport and fate of U in the subsurface. However, none of them enable quantitative predictions of the extent of U adsorption onto bacterial
cell walls under conditions not directly studied in the laboratory. Toward this end, we investigated the sorption of U by the gram positive soil bacterium *Bacillus subtilis*. This bacterium was selected for this study because its cell wall properties have been well characterized through microbiological and biochemical assays (Beveridge and Murray, 1976, 1980), and its surface and acid/base properties have been previously described by (Fein et al., 1997; Daughney and Fein, 1998). Utilizing *B. subtilis* in these experiments is also an environmentally relevant choice because this species has been isolated from uranium mine settings, and has demonstrated a strong ability to bind U (Sakaguchi, 1998).

Our objective is to test whether the site-specific surface complexation model (SCM) of Fein et al. (1997) can be used to quantify U adsorption onto *B. subtilis*. The SCM for *B. subtilis* describes specific adsorption reactions between the functional groups of the bacterial cell wall and the species in solution through mass action laws that are governed by thermodynamic stability constants. The bacterial surface deprotonation reactions for the carboxyl, phosphate, and hydroxyl functional groups are characterized by the following reactions (Fein et al., 1997):

\[
\begin{align*}
(4.1) & \quad R\text{-COO-H}^0 \Leftrightarrow R\text{-COO}^- + H^+ \quad \text{pKa} = 4.82 \\
(4.2) & \quad R\text{-PO}_4\text{-H}^0 \Leftrightarrow R\text{-PO}_4^- + H^+ \quad \text{pKa} = 6.9 \\
(4.3) & \quad R\text{-O-H}^0 \Leftrightarrow R\text{-O}^- + H^+ \quad \text{pKa} = 9.4
\end{align*}
\]

where R represents the bacterium to which each functional group is attached. The site densities and surface area utilized for this model have been determined by Fein et al. (1997). Deprotonation of the cell wall functional groups creates negatively charged
surface sites for metal adsorption according to the general reaction (written for a
generic surface functional group, A):

\[(4.4) \quad M^{m+} + R-A^- \leftrightarrow R-A-M^{(m-1)} \]

The deprotonation also leads to the development of a negative electrical potential
associated with the bacterial cell wall. This potential in turn affects the interactions of
ions with the bacterial surface sites. We can account for these effects on surface
acidity constants and metal stability constants through the following relationship:

\[(4.5) \quad K = K_{intrinsic} e^{\left(\frac{-\Delta Z F \phi_0}{RT}\right)} \]

where \(\Delta Z\) is the change in the charge of the surface species for the reaction under
consideration, \(F\) and \(R\) are Faraday’s constant and the gas constant, \(T\) is absolute
temperature, \(K_{intrinsic}\) represents the equilibrium constant referenced to zero surface
charge, and \(\phi_0\) is the electric field potential of the bacterial surface (Stumm and
Morgan, 1996). We relate the surface electrical potential to surface charge (\(\sigma\)) by a
constant capacitance double-layer model:

\[(4.6) \quad C = \frac{\sigma}{\psi} \]

where \(C\) is the capacitance of the \textit{B. subtilis} surface (8.0 F/m\(^2\)) (Fein et al., 1997).
With this approach, equilibrium constant values can be used to quantitatively predict
the extent of proton and metal adsorption onto specific bacterial surface sites over a
wide range of pH, metal concentration, and surface site concentration conditions (Fein
et al., 1997; Daughney and Fein, 1998; Daughney et al., 1998). The approach can also
successfully account for competitive adsorption effects (Fowle and Fein, 1999), and
for the competition between aqueous organic acids and the bacterial surface for
available metal cations (Fein and Delea, 1999).

In this study we document the adsorption of U onto the gram positive bacterium *B. subtilis*, and we invoke a site specific SCM to model these interactions. This study provides insights in the pH dependence, reversibility, and kinetics of U- *B. subtilis* adsorption reactions.

4.2. Experimental Procedures

The bacterial species *B. subtilis* was prepared and cultured following the procedure outlined in Fein et al. (1997), and Fowle and Fein (1999). Integrity of the cell walls after the wash procedure was monitored using microscopy and Molecular Probes - LIVE/DEAD BacLight bacterial viability kit. All solutions in this study were prepared with distilled, deionized (18 MΩ) water. Prior to each experiment, the bacteria were pelleted by centrifugation at 7500 rpm for 60 min. The mass of the pellet was measured in order to determine the concentration of surface functional groups in each experiment. Note that the weight of bacteria used in each experiment is not reported as a dried weight, but as a weight after centrifugation.

The sorption of U by *B. subtilis* was studied in 0.1 M NaClO₄ electrolyte solutions. Batch experiments were conducted at 25 ± 1°C as a function of pH, solid/solute ratio, and equilibration time. Bacteria were suspended in 0.1M NaClO₄ electrolyte, and 1000 ppm aqueous U standard was added to the bacteria-electrolyte solution to create a homogeneous parent solution of known bacterial (0.5, 1.0, 1.5 g/L) and U concentrations (0.084 mM). Aliquots of the parent solution were
transferred to the reaction vessels (acid washed polypropylene), and the pH of the suspension in each vessel was adjusted to the desired pH value using small volumes (less than 1% total volume) of standardized HNO₃ or NaOH. The pH interval of 1.5 to 5.0 was studied to focus the study on UO₂²⁺ adsorption. At higher pH values, hydroxyl and carbonate complexation of UO₂²⁺ complicates the aqueous U speciation, and was beyond the scope of this initial study. The reaction vessels were placed on a rotating rack that provided gentle (10 rpm) end-over-end agitation. The equilibrium pH was recorded and the suspension was filtered through a 0.1 μm nylon filter. The filtrate was acidified and analyzed for U content by ICP-AES (with an analytical uncertainty of ± 2%). The bacteria do not lyse, sporulate, or multiply during our experiments, and therefore cell concentrations or surface area changes do not affect our results. Control experiments followed the experimental procedure without the presence of bacteria.

Desorption experiments were conducted to determine the reversibility of U-bacteria adsorption reactions. A homogeneous parent solution of bacteria + U + electrolyte was adjusted to pH 5.0 (a pH at which nearly all of the U was adsorbed onto the bacteria) as described above. Aliquots from this parent solution were taken and adjusted to sequentially lower pH values after 2 hours of adsorption contact time. The reaction vessels equilibrated at the new pH values for 2 hours, and were sampled for U content as described above.
4.3. Results and Discussion

The cell walls of *B. subtilis* display a strong affinity for U (Figure 4.1). The control experiments revealed a minor pH-dependent systematic loss (1-10%) of U from pH 1.5-5.0, likely caused either by adsorption of UO$_2^{2+}$ onto the reaction vessels or by the formation of a uranium precipitate (no visible precipitate). All of our data were adjusted using a linear function to correct for this loss. The adjusted data is depicted in all figures. The concentration of U bound to the bacterium is strongly dependent on the solid:solute ratio, and the solution pH. Adsorption increases with increasing pH and solid:solute ratio, presumably due to the deprotonation of cell wall functional groups and the increasing number of surface reactive sites. A maximum U adsorption of 90% was observed at pH 4.9 (1.5 g bacteria/L) and the minimum adsorption of U of 12 % was observed at pH 1.7 (0.5 g bacteria/L). Remarkably, up to nearly 60% of the aqueous U (1.5 g bacteria/L) was adsorbed at solution pH values less then 2, conditions at which virtually all surface sites are fully protonated and neutrally-charged. This is in marked contrast to the adsorption behavior of other cations onto *B. subtilis*, which exhibit only small or negligible adsorption under such low pH.
Figure 4.1.

Percent adsorption of U by *B. subtilis* as a function of pH. Experiments were conducted in 0.1 NaClO₄ with $10^{-1.08}$ m U and with 0.5, 1.0, or 1.5 g bacteria/L.
Figure 4.2.

Adsorption (log molality) of U by *B. subtilis* as a function of time. Experiments were conducted in 0.1 NaClO₄ at pH 3.0 with $10^{-1.08}$ m U and with 0.5 g bacteria/L.
Figure 4.3.

The percent of total U associated with the bacterial surface after adsorption (squares) and desorption (circles), as a function of pH in 0.1M NaClO₄, with a total system concentration of U of 10⁻⁴.₀₈ m and a bacterial concentration of 1.5 g/L. Desorption began after a 2 hour period of adsorption at pH 5.0. pH was then lowered to between 2 and 5.
(Fein et al., 1997; Daughney and Fein, 1998; Fowle and Fein, 1999). The adsorption of U reaches equilibrium within 30 minutes and remains invariant through at least 24 hours (Figure 4.2). Desorption experiments (Figure 4.3), conducted at 1.5 g bacteria/L, are in excellent agreement with adsorption experiments, indicating that the adsorption of U is both rapid and reversible. Furthermore, the rapid kinetics and reversibility of U binding, in conjunction with TEM images of B. subtilis that demonstrate that U binds to the outside of the cell (Sakaguchi, 1996), strongly suggest that the loss of U from the aqueous phase in these undersaturated systems is through adsorption to the organic functional groups of the bacterial cell wall.

The experimental data were used to calculate stability constants for the U-functional group adsorption reactions. We use the program FITEQ 3.1 (Herbelin and Westall, 1994) to compare models involving different U adsorption reaction stoichiometries, and to determine the model which most accurately describes our data. Figure 4.4 illustrates the aqueous speciation of U under the experimental conditions. Due to the predominance of UO\textsubscript{2}\textsuperscript{2+} over the pH range studied, it is most likely that UO\textsubscript{2}\textsuperscript{2+} is responsible for the U uptake under the experimental conditions, and we choose to model the system using the following reaction stoichiometry:

\[
\text{UO}_2^{2+} + R-\text{AH}_x^{(x-1)} \leftrightarrow R-\text{AH}_x^{-}\text{UO}_2^{(1+x)}
\]

where A represents either a carboxyl, a phosphate, or a hydroxyl surface functional group, and x can equal either 0 or 1.
Figure 4.4.

Calculated aqueous speciation of U at 25 °C and 1 bar with aqueous concentrations of $10^{-4.08}$ M U, $10^{-3.5}$ M CO$_2$ (aq), and 0.1 M NaClO$_4$ as function of pH.
We test all possible adsorption site stoichiometries, and solve for stability constants for each proposed stoichiometry, as defined by:

\[
K^{(\eta)} = \frac{[R - AH_x - UO_2^{(1+x)}]}{a_{UO_2}^{-1} \cdot [R - AH_x^{(z-1)}]}
\]

where \(a\) represents aqueous activity and the brackets represent surface site concentrations in moles Kg\(^{-1}\) of solution. The calculation of the stability constants for the surface complexation reactions uses the equilibrium constants from Fein et al. (1997) for the acid-base properties of \(B.\ subtilis\), and those of Wolery (1992) for aqueous reactions in the Na-ClO\(_4\)-NO\(_3\)-H\(_2\)O system. The values and sources of the formation constants for the various uranium aqueous species used in this study are listed in Table 4.1.

Table 4.1. U(VI) Aqueous Phase Reactions

<table>
<thead>
<tr>
<th>Reaction</th>
<th>(\log K (I = 0.0, 25^\circ C))</th>
</tr>
</thead>
<tbody>
<tr>
<td>(UO_2^{2+} + H_2O \rightleftharpoons UO_2OH^+ + H^+)</td>
<td>-5.2</td>
</tr>
<tr>
<td>(UO_2^{2+} + 2H_2O \rightleftharpoons UO_2(OH)_{2}^\circ + 2H^+)</td>
<td>-12.0</td>
</tr>
<tr>
<td>(UO_2^{2+} + 3H_2O \rightleftharpoons UO_2(OH)_3^- + 3H^+)</td>
<td>-19.2</td>
</tr>
<tr>
<td>(2UO_2^{2+} + 2H_2O \rightleftharpoons (UO_2)<em>{2}(OH)</em>{2}^{2+} + 2H^+)</td>
<td>-5.62(^a)</td>
</tr>
<tr>
<td>(3UO_2^{2+} + 5H_2O \rightleftharpoons (UO_2)<em>{3}(OH)</em>{5}^{2+} + 5H^+)</td>
<td>-15.55</td>
</tr>
<tr>
<td>(3UO_2^{2+} + 7H_2O \rightleftharpoons (UO_2)<em>{3}(OH)</em>{7}^{2+} + 7H^+)</td>
<td>-31.0</td>
</tr>
<tr>
<td>(4UO_2^{2+} + 7H_2O \rightleftharpoons (UO_2)<em>{4}(OH)</em>{7}^{2+} + 7H^+)</td>
<td>-21.9</td>
</tr>
<tr>
<td>(UO_2^{2+} + CO_3^{2-} \rightleftharpoons UO_2CO_3^\circ)</td>
<td>9.7</td>
</tr>
<tr>
<td>(UO_2^{2+} + 2CO_3^{2-} \rightleftharpoons UO_2(CO_3)_2^{2-})</td>
<td>17.0</td>
</tr>
<tr>
<td>(UO_2^{2+} + 3CO_3^{2-} \rightleftharpoons UO_2(CO_3)_3^{4-})</td>
<td>23.63</td>
</tr>
<tr>
<td>(2UO_2^{2+} + CO_3^{2-} + 3OH^- \rightleftharpoons (UO_2)_{2}CO_3(OH)_3^{-})</td>
<td>40.82(^b)</td>
</tr>
</tbody>
</table>

The misfit of each model is quantified using the $V(Y)$ variance function in FITEQL:

$$V(Y) = \frac{\sum \left( \frac{Y_{\text{calc}} - Y_{\text{exp}}}{s_{\text{exp}}} \right)^2}{n_p \cdot n_{\text{II}} - n_u}$$

(4.9)

where $Y_{\text{calc}}$ and $Y_{\text{exp}}$ are the calculated and the experimental data, $s_{\text{exp}}$ is the error associated with the experimental data (default FITEQL 3.1 value), $n_p$ is the number of data, $n_{\text{II}}$ is the number of Group II components (total and free concentrations are known), $n_u$ is the number of adjustable parameters, and $V(Y)$ is the variance in $Y$. The $V(Y)$ value provides a quantitative measure of the goodness of fit of each model, and we use this parameter to determine the best fitting model.

Adsorption of $\text{UO}_2^{2+}$ onto deprotonated carboxyl sites is the only mechanism that can reasonably explain the pH dependence of adsorption that we observed between approximately pH 2.5 and 5.0. Models involving adsorption of $\text{UO}_2^{2+}$ onto deprotonated phosphate or hydroxyl sites offer poor fits to the pH-dependent adsorption behavior. In addition, models that included multi-dentate uranyl surface species resulted in a poor fit to the experimental data, and are not considered further. However, the pH-dependent adsorption behavior is inconsistent with the adsorption observed under low pH conditions, where we observe pH-independent adsorption, the extent of which appears to be directly related to the amount of bacteria in the system. This pH-independent behavior requires an adsorption species whose concentration does not vary over at least the pH range 1.5 to 2.5. Under these conditions, each surface functional group type is virtually fully protonated, with negligible changes in
concentration over this pH interval. Therefore, we test each possible combination, with each tested model coupling UO$_2^{2+}$ adsorption onto deprotonated carboxyl sites with adsorption of UO$_2^{2+}$ onto either a protonated carboxyl, phosphate, or hydroxyl site. The results of these calculations, for each model and bacteria concentration, are compiled in Table 4.2.

Table 4.2. Comparison of UO$_2$-B. subtilis Adsorption Models

<table>
<thead>
<tr>
<th>Bacterial Concentration</th>
<th>Model$^a$</th>
<th>log K$^b$</th>
<th>V(Y)$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5 g Bacteria / L</td>
<td>R-COOH-UO$_2^{2+}$</td>
<td>9.04</td>
<td>3.823</td>
</tr>
<tr>
<td></td>
<td>R-COO-UO$_2^+$</td>
<td>5.71</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R-PO$_4$H-UO$_2^{2+}$</td>
<td>11.88</td>
<td>1.994</td>
</tr>
<tr>
<td></td>
<td>R-COO-UO$_2^+$</td>
<td>5.432</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R-OH-UO$_2^{2+}$</td>
<td>14.08</td>
<td>2.334</td>
</tr>
<tr>
<td></td>
<td>R-COO-UO$_2^+$</td>
<td>5.50</td>
<td></td>
</tr>
<tr>
<td>1.0 g Bacteria / L</td>
<td>R-COOH-UO$_2^{2+}$</td>
<td>8.93</td>
<td>4.31</td>
</tr>
<tr>
<td></td>
<td>R-COO-UO$_2^+$</td>
<td>5.58</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R-PO$_4$H-UO$_2^{2+}$</td>
<td>11.76</td>
<td>6.186</td>
</tr>
<tr>
<td></td>
<td>R-COO-UO$_2^+$</td>
<td>5.36</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R-OH-UO$_2^{2+}$</td>
<td>DNC</td>
<td>DNC</td>
</tr>
<tr>
<td></td>
<td>R-COO-UO$_2^+$</td>
<td>DNC</td>
<td>DNC</td>
</tr>
<tr>
<td>0.5 g Bacteria / L</td>
<td>R-COOH-UO$_2^{2+}$</td>
<td>8.36</td>
<td>24.97</td>
</tr>
<tr>
<td></td>
<td>R-COO-UO$_2^+$</td>
<td>5.53</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R-PO$_4$H-UO$_2^{2+}$</td>
<td>11.83</td>
<td>27.88</td>
</tr>
<tr>
<td></td>
<td>R-COO-UO$_2^+$</td>
<td>5.303</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R-OH-UO$_2^{2+}$</td>
<td>13.23</td>
<td>28.38</td>
</tr>
<tr>
<td></td>
<td>R-COO-UO$_2^+$</td>
<td>5.439</td>
<td></td>
</tr>
<tr>
<td>Best Fitting Model$^d$</td>
<td>R-PO$_4$H-UO$_2^{2+}$</td>
<td>11.8 ± 0.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R-COO-UO$_2^+$</td>
<td>5.4 ± 0.2</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Models consider formation of surface complexes due to adsorption of UO$_2^{2+}$ onto the carboxyl, phosphate, and hydroxyl surface sites.

$^b$ Log K values are referenced to zero surface charge at 25°C.

$^c$ Variance as calculated by FITEQL. $^d$ Choice of best fitting model is based upon fit of weighted average log K values, and overall variance as calculated by FITEQL.
The results in Table 4.2 demonstrate that the best-fitting model is the one involving low pH adsorption onto protonated phosphate sites. Three lines of evidence support this conclusion: 1) for each set of bacterial concentration data, this model yields an excellent fit to the data, as demonstrated by low V(Y) values; 2) this model yields the smallest variations in the calculated log K values for each surface complex between bacterial concentration data sets; and 3) independent $^{31}$P-NMR measurements have demonstrated that UO$_2^{2+}$ binding to phosphate sites of *Mycobacterium smegmatis* occurs at pH values as low as 1.0 (Andres et al., 1994). Each dataset yields a best-fitting stability constant value for the two surface species, and we calculate the weighted average stability constant values from the three datasets, yielding a single value for each equilibrium constant that together provide the best-fitting model to all of the data. For UO$_2^{2+}$ adsorption onto protonated phosphate sites ($K_{(7)}$, with A representing a phosphate site, and x=1), the best-fitting value of log K is 11.8 ± 0.2; and for UO$_2^{2+}$ adsorption onto deprotonated carboxyl sites ($K_{(7)}$, with A representing a carboxyl site, and x=0), log K is 5.4 ± 0.2.

Figure 4.5 depicts the experimental data and model predictions for the adsorption of U onto *B. subtilis* at the three different bacteria concentrations. Each curve is in excellent agreement with its corresponding data from pH 1.5 to 2.0. Above pH 2.0, the experimental data are slightly over-predicted by the model for the 1.5 g bacteria/L system, and under-predicted for the other bacterial concentrations. However, these discrepancies are minor and within the uncertainties for the stability constants. Therefore, we conclude that these two adsorption stoichiometries, with the
Figure 4.5.

U adsorption onto *B. subtilis* as a function of pH. The curves represent the best fitting surface complexation model for each bacterial concentration, calculated using the weighted average values of the equilibrium constants of the adsorption reactions.
two calculated stability constant values, can accurately account for both the pH-dependence and the solid:solute ratio dependences of adsorption.

Although only a limited number of metal-bacteria adsorption reactions have been studied to date, recent work (Fein et al., 1997; Daughney et al., 1998) suggests that metal-carboxyl stability constants can be estimated with reasonable accuracy, using a linear free energy approach (Langmuir, 1979). We can use the results of this study to place additional constraints on these relationships. Figure 4.6 shows the relationship between aqueous metal-oxalate stability constants and those determined for metal adsorption onto deprotonated carboxyl sites on B. subtilis. The data are from Fein et al. (1997) for Al, Pb, Cd, and Cu, from Fowle and Fein (1999) for Ca, and from this study for UO$_2^{2+}$. Fein et al. (1997) determined a similar correlation between B. subtilis stability constants and those involving tiron. However, an accurate aqueous UO$_2^{2+}$-tiron stability constant value has not been measured, and so we cannot further constrain this relationship with our new data. Stability constants used for metal-oxalate complexation are those described in Martell and Smith (1977, 1982). The values were adjusted (if necessary) to zero ionic strength using the Debye-Hückel equation with parameters from Hegleson et al. (1981). Figure 4.6 illustrates an excellent correlation between aqueous and bacterial surface metal-organic complexation. The linear correlation coefficient is 0.953, a high value especially given the uncertainties in the metal-organic stability constants, the metal-carboxyl stability.
Figure 4.6.

Correlation diagram relating metal-carboxyl site (on *B. subtilis* cell wall) log stability constants to the aqueous metal-oxalate log stability constants. The linear correlation coefficient is shown for the relationship.
constants, and uncertainties in applying the Debye-Hückel equation to relatively high ionic strengths and to di- and especially tri-valent ions. This striking relationship appears to be robust, and the inclusion of the new Ca and UO$_2^{2+}$ data enables us to extend the range of the linear free energy relationship by an order of magnitude. Metal-bacteria adsorption behavior can now be estimated for a much wider range of metals.

Bacterial adsorption is likely to significantly affect the distribution, and hence mobility, of uranium in groundwater systems. Our results offer a first step toward quantifying the speciation of U in bacteria-bearing systems. The study of metal-bacteria adsorption is in its infancy, and many more interactions must be quantified before surface complexation models of realistic systems are possible. However, it is the vast range of systems that are of geologic and environmental interest that makes the surface complexation approach so powerful. To apply a surface complexation approach, a large number of isolated stability constants must be determined, either by direct measurement or by estimation techniques, but there is no other technique that yields quantitative estimations of metal distributions over a broad range of near-surface geological conditions.
References


5.1. Introduction

While weathering is often considered purely a dissolution phenomenon, mineral precipitation from aqueous solution is as important as dissolution in affecting the ultimate distribution of elements in weathering environments. Quantifying and defining the controls on mineral formation in near-surface water-rock systems is critical in order to understand processes such as global element cycling, mass transport in water-rock systems, weathering processes, and sedimentary diagenesis. Bacteria are ubiquitous in near-surface environments, and numerous laboratory and field studies demonstrate that bacteria can facilitate the formation of minerals through adsorption and nucleation reactions (Beveridge et al., 1983; Konhauser et al., 1993; Urrutia and Beveridge, 1993, 1994; Fortin and Beveridge, 1997; Fortin and Ferris,
1998; Warren and Ferris, 1998; Konhauser et al., 1997). If bacteria can enhance the
formation rates of minerals through nucleation or induce mineral formation by
changing solution chemistry either at the micro-environment scale or the bulk solution
scale, then there are profound implications for many near surface geologic systems.
For example, if bacteria within groundwater aquifers facilitate mineral formation,
then the mobility of metals may be greatly diminished, and therefore predictions of
the mass transport of metals through these systems may be overestimated. However,
despite the growing evidence that bacteria play a key role in mineral formation in low
temperature systems, our understanding of the rates and mechanisms of
biomineralization remains rudimentary.

Bacteria can directly affect the formation of minerals either through changes in
the chemical kinetics of the precipitation reaction or through changes in the saturation
state of the system (or a subset of the system) with respect to the mineral phase.
Bacteria may affect the kinetics of mineral formation by adsorbing metal cations from
solution and inducing precipitation by providing sites for the formation of stable
crystal nuclei (Fortin et al., 1997). Furthermore, if the nucleation process is due to
metal interactions with the cell wall surface functional groups, the bacteria may not
need to be metabolically active. Metal adsorption could result in the initiation of
mineral formation on dead biomass or even cell wall fragments, common constituents
of near-surface hydrologic systems. Alternatively, the bacteria may affect the
saturation state of the near-cell environment with respect to the mineral phase by
increasing aqueous metal activities in the presence of the electrified bacterial cell
wall, through proton gradients at the bacteria-water interface, or through metabolically dependent reaction mechanisms.

Laboratory and field studies have demonstrated that bacteria can assist in the formation of carbonates, sulfates, oxides, oxyhydroxides, and some silicate minerals (Fortin et al., 1997). Ferris et al. (1986) examined sediment samples from an acidic hotspring in Yellowstone National Park with electron microscopy and energy dispersive X-ray spectroscopy and discovered the remains of bacteria inside accumulations of iron and silica. The authors proposed a mechanism similar in fashion to the laboratory diagenesis study of Beveridge et al. (1983), where metal-laden cells interact with dissolved silica to form the characteristic mineralized forms of prokaryotes fossilized in sedimentary rocks. Investigating the precipitation mechanism of these Fe-SiO$_2$-Bacteria composites, Urrutia and Beveridge (1994) established that silica in the presence of *Bacillus subtilis* and Fe for several weeks enhanced the formation of fine grained silicate precipitates (including some clay type minerals). However, the study by Urrutia and Beveridge (1994) did not demonstrate if the bacteria merely acted as nucleation sites for mineral formation or if the bacterial metabolism promoted mineral formation.

Further field studies by Ferris et al. (1987), Konhauser et al. (1993), and Konhauser et al. (1997) demonstrate that complex (Fe,Al)-silicates or sulfides can be spatially associated and/or nucleated on the surface of individual bacteria colonies or biofilms in lake and river sediments in a variety of climates. These studies document the important role of bacterial surfaces in affecting mineral precipitation in various natural environments. However, the data do not enable quantitative modeling of
bacterial effects on precipitation because separation of the extent of adsorption, the role of kinetics, and the degree of saturation are not possible.

Laboratory experiments have attempted to explicitly demonstrate the role of bacteria in the formation of metal oxides, silicates, and sulfate minerals. Fortin and Ferris (1998) demonstrate significant differences in the morphologies of several mineral phases formed in a series of biotic and abiotic experiments that featured several bacterial strains in the presence of Fe, Si, and SO$_4^{2-}$. However, because of the relatively short experimental times (2 h), it is impossible to determine if this was simply a kinetic effect resulting from heterogeneous rather homogeneous nucleation of the mineral phases as the authors suggest, or a metabolically dependent result. Only one study to date (Warren and Ferris, 1998) has attempted to quantify whether the presence of bacteria can significantly alter the conditions under which metal oxides form by examining the effect of three different bacterial strains (B. subtilis, B. licheniformis, and P. aeruginosa) on hydrous ferrous oxide (HFO) formation. In this study, Warren and Ferris (1998) perform batch iron partitioning experiments conducted as a function of Fe(III) concentration and pH. These experiments demonstrate that biotic experiments all result in significantly more Fe removal from solution than do the abiotic controls, and the authors interpret this result using a surface precipitation model (Farley et al., 1985). The study demonstrates that the presence of bacterial cells in the experimental system leads to a progression from adsorption, to nucleation, and ultimately precipitation of HFO's. However, Warren and Ferris (1998) claim that their data also provide evidence that bacteria enhance not only the thermodynamic stability of hydrous ferric oxide precipitates, but the kinetics
of precipitation as well. We disagree with these claims. Because each of their experimental systems was oversaturated with respect to solid iron oxide phases, the data of Warren and Ferris (1998) can not demonstrate whether bacteria would cause iron oxide precipitation from bulk solutions otherwise undersaturated with respect to the solid phase. Without this test, changes in the thermodynamic stability of the solid phase cannot be determined. Furthermore, although the experiments clearly demonstrate enhanced removal of Fe from solution in the bacteria-bearing systems, Warren and Ferris (1998) do not explicitly account for Fe adsorption onto bacterial cell walls. Therefore, it is impossible to determine what proportion of this enhanced removal is due to cell wall adsorption as opposed to that due to enhanced precipitation kinetics in the short time interval of their experiments.

Bacterial metabolism can exert both direct and indirect effects on mineral formation. Warren et al. (1999) show that a common microbial process such as urea degradation leads to the enhanced formation of carbonates through the bacterial production of bicarbonate:

\[
\begin{align*}
(5.1) \quad \text{CO(NH}_2\text{)}_2 + 2\text{H}_2\text{O} + \text{H}^+ & \rightarrow 2\text{NH}_4^+ + \text{HCO}_3^- \\
(5.2) \quad \text{HCO}_3^- + \text{Ca}^{2+} & \rightarrow \text{CaCO}_3^{(S)} + \text{H}^+
\end{align*}
\]

Furthermore, Warren et al. (1999) describe an experimental system where urea degradation by *Bacillus pasteurei* can also promote CaCO\(_3\) formation in the presence of contaminants such as UO\(_2\)\(^{2+}\) and Sr\(^{2+}\), resulting in significant solid phase capture of the contaminants. Bacteria may also act as precipitation catalysts in metal-bacteria systems by changing solution chemistry through enzymatic reactions. Macaskie et al. (1987) show that the activity of the enzyme phosphatase liberates HPO\(_4\)\(^{2-}\) from
glycerol 2-phosphate and induces precipitation of CdHPO₄ on the surface of *Citrobacter*. A study by the same group (Macaskie et al., 1992) indicates that a similar mechanism is responsible for the formation of uranyl-phosphates on the surface of the same organism. Direct metabolism of metal ions by bacterial cells will also result in mineral formation. Fe³⁺ in iron-oxyhydroxides can be respired by dissimilatory-iron reducing bacteria under anaerobic conditions and then be excreted by the bacteria as Fe²⁺ which in turn can react with the remaining iron-oxyhydroxides to form magnetite (Bazylinski and Moskowitz, 1997). All of these studies demonstrate that bacterial metabolism can alter the solution chemistry of an experimental system and can, in turn, precipitate a mineral phase which was previously undersaturated.

The studies of Fortin and Ferris (1998) and Warren and Ferris (1998) demonstrate that bacterial cell walls are likely to promote heterogeneous nucleation of metal oxides through surface complexation reactions. This type of interaction has been demonstrated abiotically for CaF₂, MgF₂, and CaCO₃ formation on the oxides CeO₂, TiO₂, and γ-Al₂O₃ (Brown et al., 1999). In most cases, the nucleated phase has the same crystalline structure and lattice spacing as the substrate. Evidence for this phenomena in bacteria-bearing systems can be seen in the unique species-dependent morphologies of mineral phases found in the presence of bacteria (Fortin and Ferris, 1998; Warren and Ferris, 1998).

The laboratory and field studies described here demonstrate: 1) that bacteria and biofilms found in many common near-surface geologic settings have authigenic mineral phases associated with cell walls and extracellular polysaccarides; 2) that
bacterial cell walls can act as nucleation sites for metal cations thereby lowering the activation energy for mineral formation; 3) that processes associated with bacterial metabolism can lead to a change in solution chemistry, causing the conditions for the formation of a particular mineral phase to be thermodynamically more favorable. However, although several studies indicate that the mere presence of bacterial cell walls can initiate precipitation, to date no study has demonstrated conclusively that non-metabolizing bacterial cells can induce the precipitation of a mineral phase at otherwise undersaturated conditions. In this study, we test this possibility. We use non-metabolizing intact bacteria, and we conduct a number of controlled bacterial-Cu precipitation studies, measuring Cu removal from solution in both bacteria-bearing and abiotic control systems. Because we can explicitly account for Cu adsorption, and because the controls clearly define the saturation concentrations, these experiments unequivocally constrain the conditions at which mineral precipitation occurs.

5.2. Theoretical Basis for Bacterial Surface-Induced Precipitation

The concept that bacterial surfaces, like mineral surfaces, can induce surface precipitation has the potential to change our interpretation of the mechanisms of biomineralization. The study of mineral surface precipitation began with the insights of James and Healy (1972), who demonstrated through the use of electrokinetic and aqueous chemistry measurements that Co appears to precipitate onto the surface of SiO₂ and TiO₂ at undersaturated bulk solution conditions. James and Healy (1972)
proposed a thermodynamic model for surface precipitation by postulating that the electrical field at the mineral-water interfaces lowers the dielectric constant of the adjacent aqueous medium below its bulk aqueous value. This, in turn, affects the solvation energy of the ions in solution. The authors use the Born charging equation to calculate the excess free energy of the ions in the presence of the electric field, which in turn can be used to calculate the electric field induced solubility of the solid. Because the excess free energies of the ions are positive, the solubility constant for the mineral phase in the presence of the electrical field is lower than that in the bulk solution. The concept that a surface electric field can induce mineral precipitation could imply that the relatively strong electric fields associated with bacterial cell walls cause a similar effect.

The surface precipitation model of Farley et al. (1985) utilized by Warren and Ferris (1998) accounts for the non-ideal Langmurian adsorption behavior demonstrated in studies such as James and Healy (1972) with a different conceptual model. Farley et al. (1985) propose that the sorption of metal cations onto metal oxides is similar to a multi-layer B.E.T. model for gas adsorption, with a continuum existing between mono-layer surface coverage and the surface condensation of the gas. As applied to solute precipitation, this model describes a continuum between the adsorption of the metal cations (as described by surface complexation modeling) and the ultimate precipitation of a metal hydroxide phase. The model describes the continuum by invoking a solid solution that varies in composition between the original mineral phase and the metal cation that is adsorbing. The mixed solid is considered to have a lower solubility then the pure end-members, and the formation
of this new mixed phase can accurately describe the non-Langmuirian high metal cation surface coverages in these types of experimental systems.

The phenomenon of a mineral phase dissolving and a new mixed phase (consisting of both solute cations and cations from the original mineral) precipitating at what would otherwise be undersaturated conditions for a solute cation metal oxide phase has been experimentally verified by a number of recent XAFS studies. Scheidegger et al. (1997) reported spectroscopic evidence using XAFS that the adsorption of Ni(II) onto clays and aluminum oxides leads to the formation of polynuclear Ni surface complexes which are structurally related to the mixed Ni/Al mineral takovite. A similar XAFS study by Towle et al. (1997) determined that Co(II) adsorption onto Al₂O₃ leads to the formation of a mixed Co/Al phase on the surface. In this study, Towle et al. (1997) argue that if the interface is electrified, then the electrochemical potential (ζ) of all regions in thermodynamic equilibrium must be constant (Towle et al., 1997). In the bulk aqueous phase, the electric field can be assumed to be equal to zero, thereby reducing the electrochemical potential to simply the chemical potential:

\( \mu_{aq} = \mu^o + RT \ln a_{aq} \)  

(5.3)

where \( \mu^o \) is the standard molal chemical potential of the ion at the temperature and pressure of interest, \( R \) is the gas constant, \( T \) is absolute temperature, and \( a_{aq} \) is the activity of the ion in bulk solution. At the electrified interface:

\( \zeta_{int} = \mu^o + RT \ln a_{int} + zF\phi_{int} \)  

(5.4)
where $a_{\text{int}}$ is the activity of the ion at the interface, $z$ is the charge of the ion, $F$ is Faraday’s constant, and $\phi_{\text{int}}$ is the electrical potential at the interface. Towle et al. (1997) assume that the electrochemical potential at the interface is equal to that of the bulk solution, yielding the relationship:

$$\mu^\circ + RT \ln a_{aq} = \mu^\circ + RT \ln a_{\text{int}} + zF \phi_{\text{int}}$$

(5.5)

$$a_{\text{int}} = \exp\left[\frac{RT \ln a_{aq} - zF \phi_{\text{int}}}{RT}\right]$$

This relationship indicates that the activity of a charged ion at the interface differs from its activity in bulk solution. However, Towle et al. (1997) conclude that this cannot lead to the formation of a neutrally charged precipitate at otherwise undersaturated conditions. If one considers a positively charged ion interacting with a negatively charged bacterial surface, the effect of the increased activity of the cation at the interface is directly negated by the decreased activity of the anionic species, and hence the overall saturation state remains unchanged. Therefore, contrary to the model of James and Healy (1972), Towle et al. (1997) argued that the presence of a charged mineral surface does not lead to surface precipitation at otherwise undersaturated conditions. Our experiments help distinguish between these two models.

5.3. Experimental Procedures

The bacterial species *B. subtilis* was prepared and cultured following the procedure outlined in Fein et al. (1997), and Fowle and Fein (1999). Integrity of the
cell walls after the wash procedure was monitored using microscopy and a Molecular Probes - LIVE/DEAD BacLight bacterial viability kit. All solutions in this study were prepared with ultrapure (18 MΩ) water. Prior to each experiment, the bacteria were pelleted by centrifugation at 7500 rpm for 60 min. The mass of the pellet was measured in order to determine the concentration of surface functional groups in each experiment. Note that the weight of bacteria used in each experiment is not reported as a dried weight, but as a weight after centrifugation.

The removal (adsorption and precipitation) of Cu from solution in systems containing B. subtilis was measured as a function of pH in 0.1 M NaClO₄ electrolyte solutions. Cu was chosen as the representative metal for study for two reasons: (1) Cu(OH)₂(s) does not begin to precipitate until near neutral solution pH values at m m concentrations of aqueous Cu. This allows for the potential to separate adsorption and precipitation reactions through chemical equilibrium modeling. (2) The abiotic solubility of Cu(OH)₂(s) and the adsorption of Cu onto B. subtilis have been characterized in a number of recent studies (Hidmi and Edwards, 1999; Fein et al., 1997; Fowle and Fein, 1999). Batch experiments were conducted at 25 ± 1°C as a function of pH, solid/solute ratio, and Cu concentration. Bacteria were suspended in 0.1M NaClO₄ electrolyte, and 1000 ppm aqueous Cu standard was added to the bacteria-electrolyte solution to create a homogeneous parent solution of known bacterial (0.5, 1.0 g bacteria/L) and Cu concentrations (0.472 m m). Aliquots of the parent solution were transferred to the reaction vessels (acid washed polypropylene), and the pH of the suspension in each vessel was adjusted to the desired value using small volumes (less than 1% of the total volume) of standardized HNO₃ or NaOH.
The pH interval of 2 to 9 was studied to focus the study on both adsorption and precipitation of Cu in the presence of the bacteria. The reaction vessels were placed on a rotating rack that provided gentle (10 rpm) end-over-end agitation. The equilibrium pH was recorded, and the suspension was filtered through a 0.1 μm nylon filter after 24 hours of reaction time. The filtrate was acidified and analyzed for Cu content by ICP-AES (with an analytical uncertainty of ± 2%). The bacteria do not lyse, sporulate, or multiply during our experiments, and therefore cell concentrations or surface area changes do not affect our results. Control experiments followed the experimental procedure without the presence of bacteria.

Sorption isotherm experiments were conducted to determine the behavior of Cu-bacteria sorption reactions as a function of increasing aqueous Cu concentration. These tests not only examine whether a continuum exists between adsorption and precipitation (Warren and Ferris, 1998), but they also provide a sensitive test for the conditions necessary for precipitation to occur. Individual batch reactors were prepared by adding a known quantity of bacteria + Cu + electrolyte to create an experimental system with 5.0 g bacteria/L at 0.1 M ionic strength with an initial aqueous Cu concentration between 10^{-5} and 10^{-2} m. The Cu concentrations of the experiments ranged from initially undersaturated to supersaturated with respect to Cu(OH)_{2(S)}. The pH of the suspension in each vessel was adjusted to the desired pH value (5.5 or 6.8) and maintained throughout the experiment with the addition of small volumes (less than 1% of the total volume) of standardized HNO_3 or NaOH. The reaction vessels were sampled for Cu content as described above after 24 hours.

SEM samples were fixed with 2.5% glutaraldehyde in 0.1 M NaClO_4, rinsed
in 0.1 M NaClO₄, and then rinsed in 2% osmium tetroxide in 0.1 M NaClO₄. After the fixation process the samples were rinsed in 0.1 M NaClO₄ and underwent dehydration in a graded series of ethanol to 100%. The samples were then dried in a Denton DCP-1 Critical Point Dryer with liquid carbon dioxide, mounted on 10mm stubs, and sputter coated with gold-palladium (60% to 40%) by a Denton Desk II sputter coater. All samples were then viewed and photographed with a JEOL JSM-T300 scanning electron microscope using Polaroid Type 55 P/N film.

5.4. Experimental Results

The adsorption/precipitation behavior of Cu as function of pH is shown in Figures 5.1 and 5.2. The results are depicted as the percentage of Cu removed from solution, where the removal can be caused by the adsorption and/or the precipitation of Cu. Control experiments that followed the experimental procedure of the bacteria-bearing experiments demonstrate no significant loss of Cu from solution below pH 6.0. Above this pH, aqueous Cu decreases to less than 1% of the total Cu in the system due to the formation of a visible blue precipitate (CuOH₂(s), Hidmi and Edwards, 1999). Bacterial sorption experiments demonstrate enhanced removal of Cu from the aqueous phase compared to the removal observed in the abiotic controls at pH values below 6.0.
Figure 5.1.

Percentage of Cu (initial starting concentration $4.72 \times 10^{-4} \text{ m}$) removed from solution as a function of pH with (circles) and without (diamonds) bacteria present. Curve represents an independent model generated by FITEQL 3.1 based on total metal and bacteria concentrations.
Figure 5.2.

Percentage of Cu (4.72x10^{-4} m) removed from solution as a function of pH with (circles) and without (diamonds) bacteria present. Curve represents an independent model generated by FITEQL 3.1 based on total metal and bacteria concentrations.
Removal of Cu from solution in the bacteria-bearing systems increases with increasing solution pH, presumably due at least in part to the deprotonation and subsequent adsorption of Cu onto bacterial cell functional groups, as seen in previous adsorption studies (Fein et al., 1997; Fowle and Fein, 1999). However, above pH 6.0 the sorption behavior of the bacteria experiments is identical to that of the control experiments, indicating that Cu removal is occurring via precipitation from solution under these pH conditions.

The adsorption/precipitation isotherm behaviors of Cu at constant pH values are displayed in Figures 5.3 and 5.4. Figure 5.3 depicts the behavior for both 5.0 g bacteria/L and control experiments at pH 5.5. The abiotic control experiments depicted in Figure 5.3 demonstrate a minor (log molality Cu solid phase = -5.5) loss due to precipitation (or perhaps filtration) from aqueous log molality Cu equilibrium values of -4.7 to -2.8. Above these aqueous concentrations a steep incline is seen in the control data that corresponds to the onset of Cu precipitation. Bacterial experiments demonstrate Langmuirian isotherm behavior with a linear relationship between the mass of Cu sorbed and that in aqueous solution. However, the isotherm in Figure 5.3 does not exhibit the expected adsorption “plateau” that occurs when the adsorbed cations form a monolayer, but instead follows the same precipitation trend as the control experiments. Figure 5.4 depicts the adsorption/precipitation behavior of Cu at pH 6.8 as a function of equilibrium aqueous Cu concentration. At this solution pH, nearly all of the Cu added to the system for the lowest initial Cu concentrations is adsorbed to the bacterial cell functional groups.
Figure 5.3.

log m Cu removed from solution as a function log m Aqueous Cu (equilibrium Cu) with (circles) and without (squares) bacteria present after 24 hours reaction time. Curve represents an independent model generated by FITEQL 3.1 based on total metal and bacteria concentrations.
log m Cu removed from solution as a function log m Aqueous Cu (equilibrium Cu) after 24 hours reaction time. Dashed line represents the calculated saturation state based on control experiments. Curve represents an independent model generated by FITEQ 3.1 based on total metal and bacteria concentrations.
With increasing aqueous Cu concentration, Cu sorption plateaus, presumably due to a monolayer coverage of the bacterial surface. At aqueous concentrations of log molality Cu = -3.8 a vertical trend indicative of mineral precipitation is observed. After 24 hours all batch experiments which initially had higher log molality aqueous concentrations of Cu than -3.8 drop down to this same value, providing strong evidence that an equilibrium solubility is controlling the final aqueous Cu concentrations in the experimental system. The samples that define this trend also exhibited visible formation of a blue precipitate. Figure 5.5 displays SEM images of this precipitate. It is clear from Figures 5.5a and 5.5b that some of the fine grained precipitate is associated with the cell walls of *B. subtilis*. However, in Figures 5.5c and 5.5d one can also see cells without any precipitate associated with the cell wall polymers, as well as isolated Cu(OH)$_2$ clusters not associated with bacterial cells. This indicates that bulk precipitation and nucleation of the copper precipitate is likely occurring both at the bacterial surface as well as in the bulk solution concurrently.
Figure 5.5.

SEM images of Cu precipitate and *B. subtilis*. Experimental conditions of 5.0 g bacteria/L, log m Cu $-3.8$, solution pH 6.8, ionic strength 0.1 M. Scale bars represent 1 or 10 μm.
5.5. Equilibrium Modeling and Discussion

Although we observed enhanced Cu removal from solution in the bacteria-bearing experiments under pH conditions where the abiotic controls were clearly undersaturated with respect to a Cu mineral phase, this observation alone cannot be construed as evidence for enhanced precipitation. The separation of adsorption and precipitation reactions through surface complexation modeling represents the only means, other than spectroscopic approaches, to determine whether non-metabolizing bacterial cell walls can induce precipitation. Studies by Fein et al. (1997) and Fowle and Fein (1999) have determined site-specific thermodynamic stability constants for Cu surface complexes on the cell walls of *B. subtilis*, and these can be utilized to calculate the amount of Cu adsorption expected in the experimental systems of this study. Our previous studies have demonstrated that estimations of the distribution of metals and organics between the bacterial cell walls and an aqueous phase are accurate in complex systems (Fowle and Fein, 1999; Fein and Delea, 1999). If estimations of the extent of Cu adsorption that occurs in the experiments match the observations, then the agreement is strong evidence that only adsorption of Cu occurs at pH values and aqueous concentrations below the solubility of Cu(OH)$_{2(s)}$. If the observed extent of Cu removal from solution exceeds that predicted for adsorption alone, then that is evidence for the occurrence of precipitation. If such precipitation occurs under pH and Cu concentration conditions where the bulk solution is undersaturated with respect to the solid Cu phase (as determined by the abiotic experiments), then we can conclude that the bacteria cause mineral formation in
otherwise undersaturated solutions. Calculation of the equilibrium state for each chemical species in the experiments is achieved by solving the set of mass balance and mass action constraints that define the system. Mass balance constraints are applied to the system in terms of total Cu concentrations and total bacterial surface functional group site concentrations. Mass action equations relate species activities to equilibrium constants for each of the important reactions in the experimental systems. These reactions, the number of which depends on the composition of the experiment in question, include the bacterial surface deprotonation reactions for the carboxyl, and phosphate functional groups:

\[
\begin{align*}
(5.6) & \quad R\text{-COO}H^0 & \rightleftharpoons & R\text{-COO}^- + H^+ \\
(5.7) & \quad R\text{-POO}H^0 & \rightleftharpoons & R\text{-POO}^- + H^+ \\
(5.8) & \quad R\text{-O}H^0 & \rightleftharpoons & R\text{-O}^- + H^+
\end{align*}
\]

as well as cation adsorption reactions involving aqueous copper cations and each bacterial surface functional group:

\[
\begin{align*}
(5.9) & \quad R\text{-COO}^- + Cu^{2+} & \rightleftharpoons & R\text{-COO-Cu}^+ \\
(5.10) & \quad R\text{-POO}^- + Cu^{2+} & \rightleftharpoons & R\text{-POO-Cu}^+
\end{align*}
\]

where R represents the bacterium to which each functional group is attached. We neglect metal adsorption reactions with surface hydroxyl sites, although these interactions may become important at pH values above approximately 9.

Utilizing the stability constants from Fein et al. (1997) and Fowle and Fein (1999) for Cu-bacteria interactions, those of Wolery (1992) for aqueous reactions in
the Na-ClO₄-NO₃-H₂O system, and those reported by Baes and Mesmer (1976) for aqueous Cu hydrolysis reactions, we calculate the extent of adsorption expected under the experimental conditions for the bacterial systems as functions of pH and aqueous Cu concentration. As with our previous models of metal adsorption onto bacterial surfaces, we use a constant capacitance model to account for the bacterial surface electric field effects on adsorption, using FITEQL 3.1 (Herberlin and Westall, 1994) to calculate these effects.

Comparisons between the observed Cu removal and the model predictions are shown in Figures 5.1 and 5.2. The extents of adsorption and the pH dependence of adsorption are described well by the chemical equilibrium modeling of the adsorption phenomena from pH 2-6. Above pH 6 the observed extents of Cu removal increase sharply over a small pH range, while the predicted models, based solely on adsorption, remain constant at 10% (Figure 5.1) and 20% (Figure 5.2), eventually decreasing as Cu hydrolysis occurs at higher pH values (not displayed). Although the experimental data are under-predicted by the adsorption model above pH 6, the control data monitoring the bulk solubility of Cu(OH)₂(S) are in excellent agreement with the observed extents of Cu removal above this pH, indicating that the bulk solubility of the copper phase is controlling the aqueous concentration of Cu under these conditions. Below pH 6.0, the solid phase partitioning of Cu in the biotic system can be accurately described by the surface complexation model of Cu adsorption, without invoking the formation of a Cu precipitate. Therefore, our data indicate that the presence of the highly adsorptive bacterial cell walls do not measurably cause mineral formation in otherwise undersaturated solutions.
Figures 5.3 and 5.4 show the estimated concentrations of total Cu that is adsorbed onto the *B. subtilis* surface as a function of equilibrium aqueous Cu concentration. The estimated log molality of the adsorbed Cu species is shown in Figure 5.3 (at pH 5.5), and ranges from -5.0 to a plateau of -3.2. The observed extent of Cu removed from solution is in excellent agreement with this thermodynamic model for aqueous Cu concentrations below a log molality of -2.6. The model yields slightly higher (5%) predicted extents of adsorption than we observed from aqueous log molalities Cu of -4.5 to -2.6, but the differences are within the uncertainties of the model stability constants. Above these aqueous Cu concentrations, we observe enhanced Cu removal from solution, a phenomenon that cannot be explained by the adsorption model. However, the extents of Cu removal from both the abiotic and the bacteria-bearing systems again are virtually identical at these aqueous concentrations, indicating that the bulk solubility of the copper phase controls the aqueous concentration of Cu under high Cu concentration conditions.

Figure 5.4 displays the experimental data and adsorption model for Cu removal from solution at pH 6.8. The plot is significantly different in appearance from Figure 5.3 as the system is saturated with respect to Cu(OH)$_2$(S) for nearly all initial aqueous Cu concentrations. Above the initial aqueous log molality of Cu of -3.8, the addition of aqueous Cu to the system does not result in a change in the equilibrium concentration, as all of the additional Cu is incorporated into a solid precipitate. Controls were not conducted for these experiments, but rather we tested the hypothesis that Cu(OH)$_2$(S) solubility was controlling aqueous Cu concentrations by using the previous control data to calculate a solubility product (Ksp) value and to
predict the saturation state under these conditions. The saturation state is displayed in Figure 5.4 as the vertical dashed line and is in excellent agreement with the experimental data. The calculated log Ksp value of 9.25 ± 0.21 is consistent with the Hidmi and Edwards (1999) value of 9.36 ± 0.02. Their work also determined that Cu(OH)$_2$ is stable for 5-96 hours of reaction time (in a similar experimental system) after which it converts to tenorite (CuO). The observed Cu removal is in good agreement with the adsorption model from an aqueous log molalities of Cu of −7 to −5.5. From aqueous log molalities of Cu between −5.5 and −4.0 the model significantly over-predicts the amount of Cu adsorbed to bacterial cell walls. Although it is not clear why such a large misfit (10-20%) of the experimental data occurs under these conditions, there is clearly no enhanced removal of Cu, and because the observed mass of Cu adsorbed is less than the predicted, there is no enhanced precipitation. Therefore, within the uncertainty of the stability constants and the experiments, the data provide conclusive evidence that the presence of bacteria do not induce the precipitation of Cu(OH)$_2$ at otherwise undersaturated conditions. For each system studied, adsorption of Cu onto the bacterial cell walls and the abiotic precipitation of Cu(OH)$_2$ can fully account for the observed Cu removal from solution.

5.6. Conclusions

For a surface to induce precipitation at otherwise undersaturated conditions, there must be a reduction in the free energy of the solid phase. There are essentially two mechanisms by which a surface can affect the free energy of the solid phase: 1)
the surface can affect the dielectric properties of the adjacent aqueous medium which in turn could decrease the stability of free ions in the presence of the surface (James and Healy, 1972); 2) or the precipitate may be coprecipitated with components of the surface (Farley et al., 1985; Towle et al., 1999). Our experiments lend support to the interpretations of Towle et al. (1997), in that the data demonstrate that bacteria exert no discernable effect on the saturation state of Cu hydroxide under the experimental conditions. We conclude that the hypothesis of James and Healy (1972) regarding the effects of surface electric fields on mineral solubilities is not applicable to bacterial surfaces. The best-documented occurrences of surface-induced mineral precipitation involve the partial dissolution of the host mineral and subsequent precipitation of solid solution precipitates. Because this mechanism is also not applicable to bacterial surfaces, we assert that there is no equilibrium continuum between the adsorption and precipitation of Cu on bacterial cell walls, and that the two processes are separate reactions.

The use of the word “continuum” to describe bacterial adsorption and precipitation reactions suggests that there is only one overall process, with adsorption and precipitation representing two ends of the spectrum. However, our data and that of Warren and Ferris (1998) clearly show that while bacterial surfaces may serve as nucleation sites, the adsorption does not in and of itself lead to precipitation of solid phases at undersaturated conditions. Adsorption can occur under a wide range of conditions and can be best described through surface complexation modeling. Precipitation is controlled strictly by the saturation state of the bulk solution, unless the mineral formation is facilitated through enzymatic or other metabolic functions of
the bacterium. If bacterial metabolism does affect the formation of mineral phases through the development of a micro-environment around/in the cell wall, it should be noted that the resulting precipitate may be short-lived in undersaturated bulk solutions when the microorganism expires and bulk solution thermodynamics are dominant. The presence of bacteria in aqueous systems may enhance the kinetics of precipitation in oversaturated conditions, and they may alter the saturation state of bulk or micro-environment conditions through metabolic activity. Each of these processes requires further study to constrain their significance in natural settings. Our data indicate that ‘biomineralization’ does not occur in otherwise undersaturated conditions merely as a result of the presence of the adsorptive charged bacterial cell wall.
References


6.1 Contribution to Knowledge

The research presented in this dissertation has posed and answered a number of fundamental questions regarding metal-bacteria interactions including: the extent of reversibility of metal-bacteria adsorption reactions; the ability of chemical equilibrium models to quantify the competition between various metal cations and bacterial surfaces; the mechanisms for uranium binding; and effects of bacterial cell walls on precipitation. This was accomplished by means of surface complexation modeling, and laboratory based experiments.

The results of a reversibility studies demonstrate that the adsorption and desorption reactions of Ca and Cd onto *B. subtilis* are rapid, and the desorption kinetics are independent of adsorption contact time. Steady-state conditions are attained within 2 hours for all adsorption reactions studied, and within 1 hour for all desorption reactions studied. Furthermore, the extent of adsorption or desorption remains constant for at least 24 hours (and up to 80 hours for Cd). The observed
extent of desorption in the experimental systems is in excellent agreement with the amount estimated from a surface complexation model based on independently conducted adsorption experiments. Notably this series of experiments also quantifies the binding of Ca to the cell walls of *B. subtilis*. Overall, this study demonstrates that the adsorption of Cd and Ca on *B. subtilis* is a rapid, fully reversible, and hence an equilibrium process.

The competitive cation adsorption experiments in both single and double bacteria systems exhibit little adsorption at pH values less than 4. With increasing pH above 4.0, the extent of Ca, Cu, Pb and Cd adsorption increases due to the increased deprotonation of bacterial surface functional groups. In all cases studied, the estimated adsorption behavior based upon surface complexation modeling is in excellent agreement with the observations, with only slight differences that were within the uncertainties of the estimation and experimental procedures. Therefore, the results indicate that the use of chemical equilibrium modeling of aqueous metal adsorption onto bacterial surfaces yields accurate predictions of the distribution of metals in complex multi-component systems.

The cell walls of *B. subtilis* display a strong affinity for U and the concentration of U bound to the bacterium is strongly dependent on the solid:solute ratio, and the solution pH. Adsorption increases with increasing pH and solid:solute ratio, presumably due to the deprotonation of cell wall functional groups and the increasing number of surface reactive sites. Nearly 60% of the aqueous U (1.5 g bacteria/L) was adsorbed at solution pH values less then 2, conditions at which virtually all surface sites are fully protonated and neutrally-charged. This was in
marked contrast to the adsorption behavior of other cations onto *B. subtilis*, which exhibit only small or negligible adsorption under such low pH conditions (Fein et al., 1997; Daughney and Fein, 1998; Fowlie and Fein, 1999). The adsorption of U reaches equilibrium within 30 minutes and remains invariant through at least 24 hours and desorption experiments are in excellent agreement with adsorption experiments, indicating that the adsorption of U is both rapid and reversible. Of particular note, the U adsorption data require two separate adsorption reactions, with the uranyl ion forming surface complexes with the neutral phosphate functional groups and the deprotonated carboxyl functional groups of the bacterial cell wall: \( \text{R-PO}_4\text{H}^0 + \text{UO}_2^{2+} \Leftrightarrow \text{R-PO}_4\text{H-UO}_2^{2+} \) (log \( K = 11.8 \pm 0.2 \)) and \( \text{R-COO}^- + \text{UO}_2^{2+} \Leftrightarrow \text{R-COO-UO}_2^+ \) (log \( K = 5.4 \pm 0.2 \)). These new stability constants, in conjunction with other experimental and predicted stability constants, may be incorporated in surface complexation models to determine the mobility and fate of U in bacteria-bearing water-rock systems.

A systematic study of the effects of bacteria cell walls on the extent of mineral precipitation was also described. In a series of experiments in which we explicitly account for Cu adsorption and bulk solution saturation state, thereby isolating the bacterial effects on precipitation we have determined that bacterial cell walls can not induce precipitation at under-saturated conditions. These experiments were novel because I explicitly account for Cu adsorption, and because the control experiments clearly define the saturation concentrations. These experiments unequivocally constrain the effects bacteria had on mineral precipitation in our system and are in disagreement with previous unconstrained experiments (Warren and Ferris, 1998).
Bacterial adsorption is likely to significantly affect the distribution, and hence mobility, of metal cations in groundwater systems. All of these experiments were conducted over a variety of pH, metal concentrations, and numerous solid to solute ratios. Therefore the results and stability constants determined in this dissertation are applicable over a wide variety of system conditions. The study of metal-bacteria adsorption is in its infancy, and many more interactions must be quantified before surface complexation models of realistic systems are possible. However, it is the vast range of systems that are of geologic and environmental interest that makes the surface complexation approach so powerful. The results presented within this dissertation demonstrate that although the interactions between metal ions and non-metabolizing bacteria are complex and likely to have a profound effect on the mobility of metal cations in many near-surface geological systems they can be constrained through the use of equilibrium thermodynamics.

6.2 Suggestions for Future Research

The results presented in this dissertation represent a significant step towards placing controls on metal-bacteria adsorption/desorption reactions for a variety of system conditions. However these chemical equilibrium models are constrained only through bulk chemistry measurements. Recent advances in X-ray Adsorption Fine Structure (XAFS) spectroscopy should allow for the direct in situ observation of the coordination environment metal-bacterial functional group interactions (Brown et al.,
1999). Application of these techniques to the systems previously studied in this dissertation and by our group (Fein et al., 1997; Daughney et al., 1998) should refine and improve the accuracy of our surface complexation models by direct verification of the systems of interest.

The study of "biomineralization" presented in Chapter 5 represents a significant step towards debunking the mythology surrounding the effects of microorganisms on mineral formation. However, it also reveals that bacteria may significantly affect the kinetics of mineral precipitation through nucleation and potentially metabolic reactions. If bacteria can enhance the formation rates of clay minerals or induce mineral formation by changing solution chemistry either at the microenvironment scale or the bulk solution scale, then there are profound implications for many near surface geologic systems. For example, bacteria have been found to depths of several km’s within sedimentary basins (Kerr, 1997). If bacteria facilitate clay mineral formation within these systems then the porosity of the basins may be greatly diminished, and therefore the mass transport by fluids through the system may be overestimated. It has also been demonstrated that bacteria can affect the weathering rates of aluminosilicate minerals (Barker et al., 1998), but it is unclear whether bacteria play a role in the subsequent precipitation of weathering byproducts (i.e. do bacteria create a relationship between the dissolution of feldspars and the precipitation of clay minerals?). Only by constraining the aqueous geochemistry, the effects of the various cell wall components, and the metabolic state of the bacteria in laboratory experiments can we gain a thorough and quantitative understanding of the role of bacteria in these systems.
References


