THERMODYNAMIC MODELING OF METAL ADSORPTION ONTO BACTERIAL CELL WALLS: CURRENT CHALLENGES

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This chapter summarizes research that improves our understanding of metal-adsorption reactions involving bacterial cell walls. The chapter covers two general types of investigations: (1) those that aim to improve our molecular-scale understanding of metal adsorption onto bacteria, thereby improving the accuracy of thermodynamic bacterial adsorption models and (2) those that aim to improve our ability to apply thermodynamic models of metal-bacterial adsorption to complex realistic settings. The first type of research involves a range of experimental approaches, and the most common approaches are described here. The second type of research results from the need to balance the flexibility that comes with chemical sophistication in geochemical models with the practical impossibility of modeling the complexity of real systems at a molecular scale. Hybrid approaches that incorporate some degree of molecular-scale insight with testable simplifying assumptions are described and offer some hope for extrapolating our newfound molecular-scale understanding of metal–bacterial adsorption reactions to estimate mass transport in bacteria-bearing systems. The objective of this chapter is to describe both progress and remaining challenges in order to promote research that ultimately will yield accurate and flexible models of the effects of bacterial adsorption on metal distributions and speciation in natural geologic systems.

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I. INTRODUCTION

Bacteria are present in a wide range of low-temperature aqueous systems, and adsorption of aqueous metal cations onto bacterial cell walls can influence the speciation and mobility of metals in the environment (Bencheikh-Latmani *et al.*, 2003). Although the potential influence of bacterial adsorption of metals on geochemical processes has been realized for decades (Beveridge and Murray, 1976; Beveridge *et al.*, 1983), quantitative models of the effects of bacterial adsorption on metal distributions in water-rock systems still do not exist. Two major obstacles must be overcome in order to include bacterial adsorption effects in geochemical models of mass transport: (1) the molecular-scale mechanisms of metal adsorption onto bacterial cell walls must be better characterized so that accurate adsorption reactions can be formulated, and stability constants for the important metal–bacterial surface complexes can be determined and (2) commonalities between the adsorption behavior of different bacterial species must be ascertained. Although some constraints have been placed on bacterial adsorption mechanisms, the complexity of the cell wall and its associated structures make the task especially challenging. Because we know so little about adsorption mechanisms even for the relatively few species of bacteria that have been studied, it is not yet clear if adsorption mechanisms differ from species to species. The number of bacterial species of environmental and geologic interest is huge and undetermined, and if each species exhibits unique adsorption behavior, then it would be a nearly impossible task to develop quantitative models of bacterial adsorption in realistic systems.

The purpose of this chapter is to summarize the research that addresses these two obstacles and describe the avenues of research that seem most promising for overcoming these obstacles. The research that has been aimed at providing a molecular-scale understanding of bacterial cell wall adsorption includes studies from a wide range of experimental and modeling approaches. Each approach has strengths and limitations, and each approach provides a different type of constraint on the adsorption mechanisms and on the thermodynamic properties of the cell-wall surface complexes. There are relatively few studies that have addressed the challenges involved in extrapolating mechanistic investigations involving single bacterial species to quantifying bacterial adsorption of metals in complex realistic settings. This chapter summarizes a body of work that suggests that adsorption onto a wide range of bacterial species can be successfully modeled using a single set of stability constants for proton and metal-binding reactions. The study of adsorption mechanisms and metal adsorption in realistic settings is certainly far from complete, and this chapter is meant to assess our current state

of knowledge with the hope of inspiring research that will improve our understanding of these complex biogeochemical reactions even more.

The studies that are reviewed here involved nonmetabolizing bacterial cells only. These investigations not only serve as a baseline for studies of adsorption onto actively metabolizing cells but they also mimic the passive nonmetabolic adsorption that likely dominates in oligotrophic subsurface conditions. High rates of bacterial metabolism can be induced in engineered systems through the introduction of high concentrations of electron donors, and similar conditions can be found in some geologic settings. However, in general bacteria exist in the subsurface under nutrient-poor conditions (Ehrlich, 1996), and in these settings, passive cell wall adsorption will be significantly more important than adsorption onto cell walls of actively metabolizing cells.

II. MECHANISTIC STUDIES OF CELL WALL ADSORPTION

A. Partitioning Relationships Versus Surface Complexation Modeling

Metal adsorption onto bacterial surfaces has been studied extensively over the past 25 years. However, much of this previous work has been either qualitative or has quantified adsorption using a bulk-partitioning approach, making it impossible to estimate the effects of bacteria on mass transport under conditions different or more complex than those studied. Because of the complexities associated with natural systems, partitioning adsorption models are relatively simple to apply because they do not require a detailed understanding of the nature of the surfaces or adsorption mechanisms involved. That is, the extent of adsorption can be measured directly on a sample of material from the field, and a bulk partition coefficient can be determined, which describes the distribution of the species of interest between the bacterial surface and the other phase, or phases of interest. These are useful measurements, and they can be successful in describing adsorption/desorption processes for the site of interest. However, partition coefficients are applicable only to the conditions at which they were determined. Because the dominant adsorption reaction can change as a function of changing conditions, partition coefficient values can vary significantly as a function of pH, ionic strength, mineralogy, type of bacterial surface, aqueous solute speciation, and solute:surface area ratio (Bethke and Brady, 2000; Koretsky, 2000).

Over the past few years, experimental studies have demonstrated that surface complexation modeling can be used to account for metal adsorption onto bacterial surfaces (see Fein, 2000, for a review). This site-specific approach requires a thorough and comprehensive understanding of the cell wall-surface chemistry. Metal adsorption onto bacterial cell walls occurs because the cell walls contain a variety of organic functional groups, which display electrostatic and chemical affinities for positively charged metals. The most common organic functional groups present on the macromolecules present within bacterial cell walls are carboxyl, hydroxyl, and phosphoryl sites, with amino groups present to a lesser extent (Beveridge and Murray, 1980). Electrophoretic mobility data indicate that, typically, cell walls are uncharged at low pH (below approximately pH 2 in most cases), but they become increasingly negatively charged at higher pH values (Ams *et al.*, 2004; Claessens *et al.*, 2004; Fein *et al.*, 2005; Harden and Harris, 1953). Surface complexation modeling ascribes the electronegativity of the cell wall to deprotonation reactions involving cell wall organic functional groups.

$$R - AH^0 \Leftrightarrow R - A^{-1} + H^{+1} \tag{1}$$

where R represents the bacterial cell wall macromolecule to which each functional group type, A, is attached. Under this formalism, adsorption of aqueous metal cations (M^{m+}) onto deprotonated surface sites on the cell wall is represented as:

$$x(M^{m+}) + y(R - A^{-1}) \Leftrightarrow (R - A)_y(M)_x^{(xm-y)^+} \tag{2}$$

where x and y represent the stoichiometric coefficients and must be determined experimentally. Surface complexation modeling is so named because it explicitly accounts for the adsorbed metal as a "surface complex" or a distinct thermodynamic species with a fixed stoichiometry. The surface complex, like its cousin the aqueous complex, has a thermodynamic stability described by the equilibrium constant, K, which, for example, is here written for equilibrium (2):

$$k = \frac{[(R - A)_y(M)_x^{(xm-y)^+}]}{(a_{M^{m+}})^x[(R - A^{-1})]^y} \tag{3}$$

where brackets represent concentration in terms of moles of sites per kg of solution, and a represents the thermodynamic activity of the subscripted species.

Surface complexation models require a detailed understanding not only of the speciation of the aqueous species and surfaces involved but also of the adsorption/desorption mechanisms. A surface complexation model treats

the adsorbed metal as another species whose stability can be quantified with an equilibrium constant. By knowing the equilibrium constant values for each of the important equilibria in a system, the distribution of metals between various reservoirs (in solution, on mineral or bacterial surfaces) can be explicitly calculated. The equilibrium constants, which describe the extent of adsorption in surface complexation models, are invarient with respect to most of the parameters that affect partition coefficients. Therefore, the equilibrium constants determined in systems, which isolate specific adsorption reactions, can be combined in computational models of more complex systems. Modeling the effects of bacterial adsorption on mass transport in geologic settings using a surface complexation approach requires knowledge not only of the absolute concentrations of the dominant bacterial species but also a knowledge of the reaction stoichiometries and deprotonation and metal-binding constants for the functional group types on the bacterial cell walls.

B. Constraints on Bacterial Cell Wall-Protonation Reactions

The choice of the most appropriate surface complexation model for cell wall adsorption is complicated by the complex chemical reactivity of bacterial surfaces. An aqueous organic acid, such as acetic acid, exhibits proton-buffering capacity over a relatively narrow pH range. This range is limited to the pH region in which significant concentrations of both the protonated and deprotonated species exist simultaneously, a pH range of 1–2 pH units to either side of the acidity constant for the acid. The organic acid functional groups on bacterial cell walls, however, exhibit an almost continuous buffering capacity over a wide pH range from, typically, approximately pH 2–10. There is considerable uncertainty whether this protonation/deprotonation behavior is best explained due to three to four functional group types that exhibit discrete pK_a values (Cox *et al*., 1999; Fein *et al*., 1997, 2005; Haas *et al*., 2001), or whether a smaller number of (one or two) functional groups with a distribution of pK_a values are responsible (Martinez *et al*., 2002; Plette *et al*., 1995). A range of experimental approaches has been used to constrain the nature of the protonation reactions on bacterial cell walls. Each approach has strengths and limitations, yet an unambiguous understanding of cell wall-protonation behavior remains elusive.

Similarly, there is considerable uncertainty concerning the best approach for accounting for the effects of the surface electric field associated with bacterial cell walls. Fein *et al*. (Daughney and Fein, 1998; Fein *et al*., 1997; Yee and Fein, 2001) have used an approach analogous to that applied to mineral surface electric fields, employing a constant capacitance model

(CCM) in conjunction with the Boltzmann equation (Stumm and Morgan, 1996). Plette *et al.* (1995, 1996), Martinez *et al.* (2002), and Yee *et al.* (2004b) utilize Donnan models, which unlike the constant capacitance model, do not assume a planar charge at the bacterial surface but rather assume a uniformly distributed charge throughout the cell wall volume. A number of models are possible because there are few data that constrain the properties of the electric field associated with bacterial cells. One approach that appears to be promising for probing the nature and extent of electrostatic interactions with cell walls is atomic force microscopy (AFM) (Camesano and Logan, 2000; Lower *et al.*, 2000). For example, Camesano and Logan (2000) used AFM measurements to demonstrate that electrostatic repulsion forces between bacterial polymers and the AFM tip are larger and extend over longer distances (up to hundreds of nanometers) than predicted by DLVO theory, and that pH effects dominate over ionic strength effects. These types of measurements are crucial in order to formulate a theory that explains the effects exerted by bacterial cell wall-electric fields on the distribution and speciation of protons and metals on the cell wall.

Most of the information on cell wall-protonation behavior has come from potentiometric titrations of suspensions of bacterial cells. In these experiments, the change in pH of a bacterial suspension is measured after additions of a known volume of acid or base. Essentially, these are proton-adsorption experiments that measure the concentration of free protons (solution pH) for a condition at which the total number of protons added to the system (the concentration of acid/base) is known. These measurements are powerful tools for determining unequivocally the total number of protons that adsorb onto bacterial cell walls. Although titration data yield rigorous constraints on total proton-active site concentrations on the cell wall, bacterial surface charge properties and mechanisms responsible for protonation behavior cannot be uniquely determined from potentiometric titrations alone (Fein *et al.*, 2005).

A growing number of potentiometric studies of the protonation of bacterial cell walls have been conducted (Cox *et al.*, 1999; Daughney and Fein, 1998; Fein *et al.*, 1997, 2005; Haas *et al.*, 2001; Martinez *et al.*, 2002; Ngwenya *et al.*, 2003; Plette *et al.*, 1995; Sokolov *et al.*, 2001; van der Wal *et al.*, 1997; Wightman *et al.*, 2001; Yee and Fein, 2001; Yee *et al.*, 2004b). These studies are consistent in that each indicates that functional groups on bacterial surfaces are proton active over a broad pH range, including down to pH values of as low as 2 and up to pH values as high as at least 10. Studies of protonation behavior of the cell wall-functional groups is complicated outside this pH range due to structural damage that is sustained by the cell wall when exposed to acidic or basic solution conditions (Borrok *et al.*, 2004c). The buffering intensity of the bacterial cell walls is characterized by two broad peaks of maximum buffering intensity: one at pH 4–5 and one above pH 8.5 (Fein *et al.*, 2005).

Despite the general agreement between data-sets that measure bacterial cell wall-protonation behavior with titrations, these studies apply a wide range of modeling approaches to interpret the potentiometric data. For example, Plette *et al.* (1995) used a three-site Langmuir–Freundlich model (LF), coupled with the Gibbs–Donnan shell model, to account for their experimental acidimetric titration measurements of *Rhodococcus erythropolis* A177. Fein *et al.* (1997) conducted potentiometric titrations of *Bacillus subtilis* and modeled the results with three discrete cell wall-functional groups, using a constant capacitance model. Cox *et al.* (1999), in similar experiments, but using a nonelectrostatic (neglecting the effects of the double layer) linear programming method modeling approach (Brassard *et al.*, 1990), modeled titration data using five discrete binding sites. Martinez *et al.* (2002) interpreted potentiometric titration data for *B. subtilis* and *Escherichia coli* by considering a set of equally spaced ($\Delta pK_a = 0.2$) pK_a values and using the Gibbs–Donnan shell model to account for electrostatic effects, finding evidence for four types of bacterial surface functional groups. Fein *et al.* (2005) demonstrated that a range of models, from a discrete site nonelectrostatic model (NEM) to a Langmuir–Freundlich continuous pK_a distribution approach, can equally well describe a single set of potentiometric titration data (Fig. 1). As the range of titration models that can be found in the literature demonstrates the buffering behavior, and intensity can be modeled either with a relatively large number (four) of discrete proton-active sites that are unaffected, or only minimally affected, by surface electric field effects, or with fewer sites that are proton active over a wider pH range than

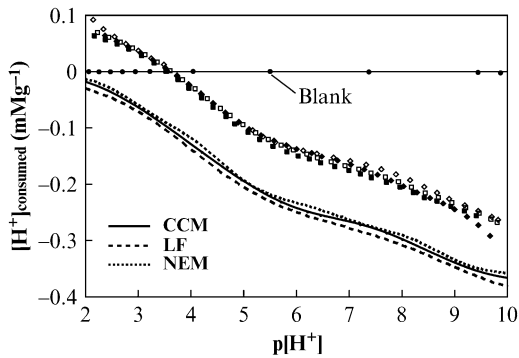

Figure 1 Potentiometric titration data of 75–150 g l^{-1} (wet weight) *B. subtilis* in 0.1 M $NaClO_4$. The data are calculated with $[H^+]_{consumed} = 0$ at the pH of suspension. The model curves are calculated taking into account T_H^0, and hence are displaced downward by this amount. The three curves are from a CCM, a LF, and a NEM. Within error, each of these modeling approaches provides a reasonable fit to the experimental data. Reprinted from Fein *et al.* (2005). *Geochim. Cosmochim. Acta*, **69**, 1123–1132, with permission from Elsevier.

the discrete sites in the "nonelectrostatic" models. The wider pH range of influence can be caused either by the effects of a bacterial surface electric field, or it may be the result of "site" heterogeneity, or it may result from a combination of the two effects.

Additional constraints on the molecular-scale protonation mechanisms exhibited by bacterial cell walls have been provided by infrared spectroscopic investigations. Infrared spectroscopy has been used as a direct determination of the identity of bacterial functional group types, with studies demonstrating the importance of carboxyl, phosphoryl, hydroxyl, and amino groups (Benning *et al.*, 2004a,b; Dittrich and Sibler, 2005; Jiang *et al.*, 2004; Yee *et al.*, 2004a). Most of the studies use infrared spectroscopy as a qualitative tool to identify functional groups present on cell walls. However, Yee *et al.* (2004a) and Jiang *et al.* (2004) also use infrared spectroscopy to investigate the pH dependence of the protonation state of cell wall-functional groups. The number of pH conditions studied in each investigation is limited, but the coverage is sufficient to demonstrate an increase in the vibrational frequencies of deprotonated carboxyl groups with increasing pH. The ability to discern protonated from deprotonated functional groups using infrared spectroscopy suggests that more detailed examinations of the pH dependencies could possibly be used to provide constraints on the mechanisms of proton adsorption and to distinguish between discrete or continuously distributed acidity constants for functional group sites.

Calorimetric measurements of bacterial surface protonation reactions offer another relatively untapped resource for constraining proton adsorption mechanisms. Calorimetry measurements of proton adsorption onto bacterial cell walls not only can provide rigorous constraints on the extent of bulk proton adsorption but also interpretation of these data using a surface complexation modeling approach can yield site-specific enthalpies and entropies of proton adsorption onto the bacterial surface functional groups. These data can be interpreted to yield information on proton coordination environment, as well as the temperature dependence of the protonation reactions. Gorman-Lewis *et al.* (2006) have conducted the only calorimetric study of bacterial surface protonation to date. Their results (Fig. 2) indicate that the protonation reaction is exothermic, with calculated site-specific entropies of protonation that are relatively small. This suggests that the functional groups on the bacterial surface behave more like multifunctional organic acids with nearby proton-active sites rather than simple monofunctional acids with a single isolated functional group. Hydrogen bonding between protonated and deprotonated sites likely occurs as reflected by the relatively small entropies of protonation. The calorimetry results suggest that multifunctional organic acids likely represent better analogues than monofunctional acids for modeling the acidity behavior of the functional groups present on bacterial surfaces.

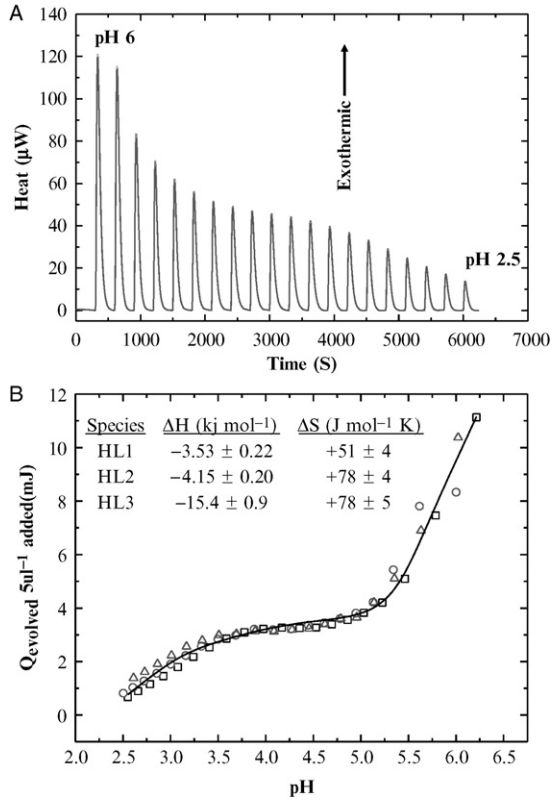

Figure 2 (A) Raw data from a typical low-pH proton adsorption calorimetric titration experiment, showing heat associated with adsorption of protons onto functional groups on the bacterial cell wall of *B. subtilis*. Data show continuous uptake of protons, and that the cell wall-functional groups are not fully protonated even under the lowest pH conditions of these experiments. (B) Corrected heat evolved from three low-pH proton adsorption calorimetric titration experiments involving *B. subtilis*, with the curve representing the best fit to the data assuming three types of protonation sites each with their own discrete pK_a value. Each figure indicates that protonation reactions are exothermic with heats comparable to those associated with protonation of multifunctional organic acids. The calculated site-specific enthalpies and entropies of protonation are listed in (B), and the low values for ΔS suggest hydrogen bonding between functional group sites. Reprinted from Gorman-Lewis *et al.* (2006, in press) *Geochim. Cosmochim. Acta*, with permission from Elsevier.

There are two main types of studies of bacterial surface protonation reactions: ones like potentiometric titration experiments that provide primarily bulk proton adsorption information and ones like infrared spectroscopy and calorimetry that yield information on proton adsorption mechanisms.

The first type of experiments provide rigorous constraints on total proton-active functional group site concentrations and the data are the easiest to use to calculate acidity constants and specific site concentrations for individual functional groups on the bacterial surface. However, as discussed earlier, titrations offer little information on molecular-scale protonation mechanisms. Conversely, infrared spectroscopy and calorimetry have the potential to significantly enhance our understanding of bacterial cell wall reactivity, but the signal can be difficult to interpret unequivocally, and the data are not geared toward constraining thermodynamic parameters of the bacterial surface species. Clearly, better constraints on mechanisms of protonation of bacterial cell wall-functional groups will come from a combination of these and other approaches.

C. Constraints on Mechanisms of Metal Adsorption onto Bacteria

Bulk metal-adsorption experiments, conducted as a function of pH and solute:sorbent ratio, provide constraints on the stoichiometries and thermodynamic stabilities of the important metal–bacterial surface species. A number of studies over the past decade have used surface-complexation modeling of bulk metal-adsorption measurements to determine binding mechanisms for metals onto bacterial cell walls (Fein *et al.*, 1997; Martinez and Ferris, 2001; Plette *et al.*, 1996). Bulk adsorption studies have demonstrated that metal and proton adsorption onto bacterial cell wall-functional groups typically is rapid and reversible (Daughney and Fein, 1998; Fowle and Fein, 2000). Furthermore, surface complexation modeling can successfully account for metal competition, pH effects, and changing solute:sorbent ratio on metal distributions in bacteria-bearing systems. Fowle and Fein (1999) demonstrated that stability constants for metal–bacterial surface complexes that are derived from single metal, single bacterial species experiments can be used to accurately estimate the extent of adsorption that occurs in more complex mixed systems. In addition, stability constants for bacterial surface complexes that involve metals that have not been studied directly can be estimated using linear free energy predictive approaches that quantify relationships between metal–bacterial stability constants and stability constants for metals complexed to aqueous organic acid anions (Fein *et al.*, 2001).

Although bulk adsorption experiments offer only circumstantial evidence of the dominant adsorption reactions, these measurements can provide constraints on the identity and thermodynamic stability of important metal–bacterial surface complexes. For example, Gorman-Lewis *et al.* (2005) measured aqueous uranium adsorption onto *B. subtilis* as functions of pH, solid:solute ratio, and dissolved CO_2 and Ca concentrations.

In oxygenated, CO_2-rich systems, negatively charged uranyl–carbonate complexes dominate the aqueous uranium speciation, and it is commonly assumed that these complexes exhibit negligible adsorption onto negatively charged surfaces such as bacteria. However, Gorman-Lewis *et al.* (2005) observed extensive uranium adsorption onto the bacterial surface, including under conditions where negatively charged aqueous uranyl–carbonate complexes dominate the aqueous uranium speciation (Fig. 3). Thermodynamic modeling of the data suggests that uranyl–hydroxide, uranyl–carbonate, and calcium–uranyl–carbonate species each can form stable surface complexes on the bacterial cell wall, results that could dramatically alter predictions of uranium mobility in near-surface environments. Similarly, Markai *et al.* (2003) interpreted bulk Eu(III) adsorption measurements using a surface complexation modeling approach, demonstrating that Eu(III) binds with both carboxyl and phosphoryl functional groups on *B. subtilis* bacterial cell walls. The adsorption data constrain both the reaction stoichiometry and the thermodynamic stabilities of the important Eu-bacterial surface complexes, parameters that are crucial for modeling the effects of bacterial adsorption on the mobility of Eu in bacteria-bearing geologic systems.

Although bulk adsorption experiments represent a powerful approach for quantifying stability constants of metal-adsorption reactions, the data provide relatively weak circumstantial constraints on the molecular-scale mechanisms responsible for the metal adsorption. More rigorous constraints on adsorption mechanisms are offered by a range of complementary

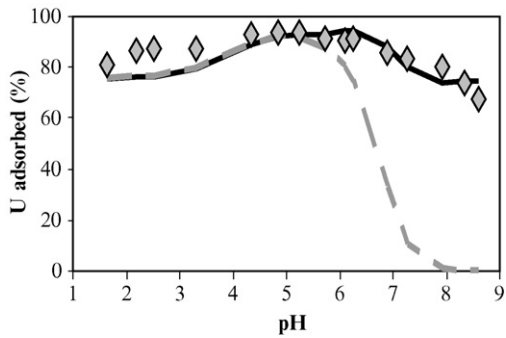

Figure 3 Measured adsorption of aqueous U(VI) onto *B. subtilis* bacterial cells (0.5 gm wet mass l^{-1}) suspended in solutions in equilibrium with atmospheric CO_2. Dashed curve represents a thermodynamic model prediction for the extent of adsorption assuming that uranyl–carbonate complexes do not adsorb and remain in solution; solid curve represents the best-fitting model constructed assuming that uranyl–carbonate bacterial surface complexes form. Data suggest that significant adsorption of aqueous uranyl–carbonate complexes onto the bacterial cell wall can control U speciation and mobility in near-neutral waters. Reprinted with permission from Gorman-Lewis *et al.* (2005). Copyright 2005, American Chemical Society.

experimental approaches, and a number of these approaches have been applied successfully to examine metal–bacterial adsorption reactions. X-ray absorption spectroscopy (XAS) has been used to constrain the binding environment for metal cations on cell wall-functional groups (Boyanov *et al*., 2003; Francis *et al*., 2004; Hennig *et al*., 2001; Kelly *et al*., 2002; Panak *et al*., 2002a,b; Sarret *et al*., 1998; Songkasiri *et al*., 2002; Templeton *et al*., 2003). Spectroscopic data provide more direct evidence of the metal-coordination environment than do bulk adsorption measurements. However, due to the complexity of the cell wall-binding environments, and due to the fact that XAS yields averaged information on all binding environments sampled by the technique, the experimental results from XAS can be complex and difficult to interpret. For example, although XAS has been used successfully to demonstrate the importance of carboxyl and phosphoryl binding sites for U and Cd on the bacterial cell wall of *B. subtilis*, significant uncertainties exist regarding the metal:ligand stoichiometry involved in binding (Boyanov *et al*., 2003; Kelly *et al*., 2002). Although the XAS data from these studies do not conclusively determine whether binding sites on bacterial cell walls exhibit discrete or continuous acidity constants, the data do require at least three distinct binding sites over the pH range of 3.5–7.0, and the data do not point to the type of binding complexity that might be expected for a cell wall on which functional groups deprotonate continuously over a wide pH range.

Calorimetric measurements of metal binding onto bacterial surfaces, interpreted using a surface complexation modeling approach, can be used to infer site-specific enthalpies and entropies of metal adsorption. Weppen and Hornburg (1995) used flow calorimetry to compare the metal sequestration capability of cell wall suspensions harvested from a range of bacterial cells. Their measured bulk enthalpies of metal adsorption (for Cd, Cu, Zn, Pb, Mg, Ca, Sr, and Ba) were all modestly endothermic (+3 to +18 $kJmol^{-1}$), and in the expected range for divalent cations coordinated with anionic oxygen ligands. However, Weppen and Hornburg (1995) did not correct for the heat associated with protonation reactions, and used a bulk-partitioning approach instead of a site-specific surface complexation model to quantify metal adsorption. Gorman-Lewis *et al*. (2006) used calorimetric data, in conjunction with surface complexation models of the protonation of the bacterial cell wall, to produce site-specific enthalpies and entropies of Cd adsorption onto the cell wall of *B. subtilis*. The calculated entahlpies of Cd adsorption are typical for Cd complexation with anionic oxygen ligands; the entropies are indicative of inner sphere complexation by multiple ligands; and the calorimetry results also constrain both the stoichiometry of the adsorption reactions and the temperature dependence of the stability constants for the dominant metal–bacterial surface complexes.

The need to elucidate mechanisms responsible for metal adsorption onto bacterial cell walls has led to the use of other molecular-scale experimental approaches. For example, time-resolved laser-induced fluorescence spectroscopy (TRLFS), like XAS, can characterize the binding environment of fluorescent elements attached to bacterial cell wall-functional groups. Texier *et al.* (2000) used TRLFS to determine that Eu binding onto *Pseudomonas aeruginosa*, a gram-negative bacterial species, occurs primarily through carboxyl and phosphoryl binding at pH 6. Similar results were obtained by Markai *et al.* (2003), who studied Eu adsorption onto the gram-positive species *B. subtilis* using TRLFS, and found carboxyl groups primarily responsible for Eu binding at pH 5, with phosphoryl groups becoming important at pH 7. The TRLFS data also provide some constraints on the metal:ligand ratio for the dominant adsorption reactions. In contrast to the mixed carboxyl and phosphoryl binding observed in the studies by Texier *et al.* (2000) and by Markai *et al.* (2003), Panak *et al.* (2000, 2002b) used TRLFS, in conjunction with EXAFS, to demonstrate that U(VI) forms inner sphere complexes only with phosphate groups on cell walls of a number of *Bacillus* species at pH 4.5–5.0.

Bulk adsorption measurements and a range of complementary experimental approaches have shed light on the molecular-scale mechanisms responsible for metal adsorption onto bacterial cell walls, however, considerably more work must be conducted to yield a complete understanding of these important geochemical reactions. Our understanding of the protonation behavior of cell wall-functional groups is rudimentary, and a range of protonation models can be invoked to explain observed protonation behaviors. Although spectroscopic and calorimetric investigations of metal binding onto bacterial cells can provide tighter constraints on the cell wall reactivity, there have been relatively few detailed studies to date. Due to the growing number of experimental and theoretical approaches, the next 5 years of research is likely to yield dramatically improved and detailed understandings of the mechanisms that control metal binding onto bacterial cell walls. One of the most important challenges that must be addressed is to better determine the nature and protonation behavior of the proton-active sites on the cell wall. Although potentiometric titrations offer rigorous constraints on total site concentrations, the data do not effectively distinguish between sites that exhibit discrete deprotonation behavior and sites that influence each other and exhibit relatively continuous deprotonation over a wide pH range. Furthermore, future research should include investigations into bacterial surface electric field effects and how to characterize them. Some attempts at this have been made using mineral surface electric field models. However, the three-dimensional nature of the cell wall and its constituent macromolecules suggests that a more sophisticated approach may be required (Wasserman and Felmy, 1998; Wasserman *et al.*, 2000). A number of

spectroscopic approaches have been used to study metal binding onto bacterial cell walls. Although much has been learned, our mechanistic understanding of these reactions is still rudimentary and considerably more research can be conducted in order to elucidate the dominant adsorption reaction stoichiometries, and hence binding environments, responsible for metal complexation on bacterial cell walls.

III. CHALLENGES IN APPLYING SURFACE COMPLEXATION MODELS TO REAL SYSTEMS

Laboratory and field studies have demonstrated that bacterial cell walls efficiently adsorb a variety of aqueous metal cations and organic molecules (Baughman and Paris, 1981; Beveridge and Murray, 1976, 1980; Gonçalves *et al*., 1987; Harvey and Leckie, 1985; Konhauser *et al*., 1993). Under some conditions, bacteria are mobile in the subsurface (Allen and Morrison, 1973; Bengtsson and Lindqvist, 1995; Gannon *et al*., 1991; Harvey *et al*., 1989, 1993; Johnson and Logan, 1996; McDowell-Boyer *et al*., 1986), and they can enhance contaminant mobilities through adsorption of contaminants onto bacterial cell walls (Champ *et al*., 1984; Champlin and Eichholz, 1976; McCarthy and Zachara, 1989). Bacteria can also be immobile due to biofilm formation, bacterial adsorption onto mineral surfaces, and/or due to bacterial straining by the rock matrix. When immobile, metal adsorption onto the bacteria is likely to enhance metal contaminant retardation (Malard *et al*., 1994; Matthess and Pekdeger, 1985). Whether bacteria are mobile or immobile, when they represent a significant portion of the solid surface area with which groundwater comes into contact, bacteria-water-rock interactions must be quantitatively accounted for in order to accurately assess mass transport in realistic subsurface environments. Most experimental studies of metal adsorption onto bacterial cell walls involve only a single bacterial species. There are a number of challenges that must be addressed in order to extrapolate these findings from studies involving individual bacterial species to realistic settings that include a myriad of species.

One of the most problematic obstacles to the application of surface complexation modeling to realistic systems is the determination of adsorption site concentrations in geologically and biologically complex systems (Davis *et al*., 1998; Payne *et al*., 2004). A bacteria-bearing natural system can contain a large number of different bacterial species, and the number of species of environmental interest is huge and undetermined. If bacterial surfaces are unique and if each species exhibits unique adsorption properties, then it would be a Herculean task to determine the binding site concentrations and binding constants for each bacterial species of environmental interest.

Experimentation on dozens of different bacterial species would be necessary just to describe the metal behavior in a single environment. If bacterial species differ significantly from each other in their adsorptive behaviors, then the complexities of realistic systems would make it virtually impossible to model metal speciation and distribution on a molecular scale due to practical computational limitations. Conversely, application of too simple a model, which does not account for surface and solute speciation changes with changing subsurface conditions, can lead to inaccurate predictions of metal speciation and mobility (Bethke and Brady, 2000; Koretsky, 2000).

A potential solution to this dilemma arises from the observations that a number of bacterial species exhibit similar extents of metal adsorption (and similar proton and metal-binding constants) as determined in laboratory experiments using individual pure strains of bacteria (Daughney *et al.*, 1998; Kulczycki *et al.*, 2002; Ngwenya *et al.*, 2003; Small *et al.*, 1999). Yee and Fein (2001) hypothesized that a common mechanism of adsorption exists for a wide range of bacterial species, and they conducted potentiometric titrations and Cd–bacteria adsorption experiments using a range of gram-positive and two gram-negative bacterial species. Yee and Fein (2001) observed one common adsorption edge (Fig. 4 for the wide variety of bacterial species studied, suggesting that the structures that give rise to metal and proton adsorption onto bacteria are common over a wide range of bacterial species. Based on the universality of the Cd-adsorption edge, Yee

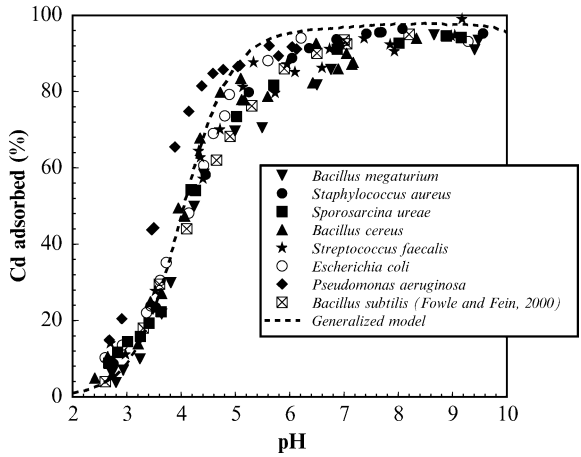

Figure 4 Cd adsorption onto pure cultures of individual gram-positive and gram-negative bacterial species. Each data point represents individual batch experiments with $10^{-4.1}$ M Cd and 1.0 g l^{-1} (dry wt.) bacteria. The dashed curve represents the adsorption behavior calculated using the average parameters given in Yee and Fein (2001). Reprinted from Yee and Fein (2001). *Geochim. Cosmochim. Acta* (2001) **65**, 2037–2042, with permission from Elsevier.

and Fein (2001) proposed that averaged thermodynamic parameters and site concentrations can be used to predict Cd adsorption onto bacterial surfaces for all bacterial species over a wide range of conditions, regardless of which species or groups of species are present in the system of interest.

The hypothesis of universal bacterial adsorption behavior has been supported by a number of subsequent experimental studies. Yee and Fein (2003) tested the universality of bacterial adsorption by measuring metal-adsorption behaviors of a mixture of 5 different gram-positive species, a mixture of 5 different gram-negative species, and a mixture of the 10 gram-positive and gram-negative bacteria, noting that all mixtures that were tested exhibited nearly identical extents of adsorption. Jiang *et al.* (2004) used attenuated total reflectance Fourier-transform infrared spectroscopy of both gram-positive and gram-negative bacterial species to demonstrate that the infrared spectra of both gram-positive and gram-negative bacteria are similar and exhibit similar variations as a function of changes in pH. The similarity in spectra for this range of bacteria suggests a similarity in binding environment for metals, and this observation supports a universal adsorption behavior that is rooted in similar cell wall-functional group chemistries. Borrok *et al.* (2004a) measured H^+ and Cd adsorption onto bacterial consortia from a range of natural environments. Their results indicate that the consortia adsorb similar extents of protons and Cd, and that the adsorption onto all of the consortia can be modeled using a single set of stability constants (Fig. 5.). In addition, Borrok *et al.* (2005) compiled all available potentiometric

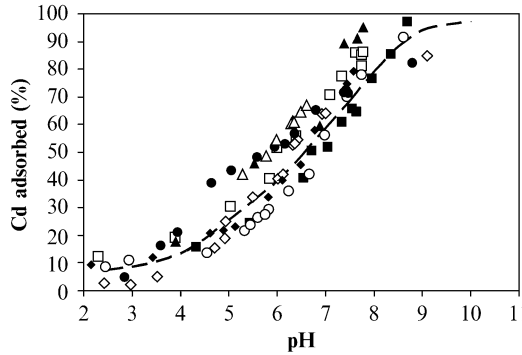

Figure 5 Data from Borrok *et al.* (2004a). Cd adsorption onto bacterial consortia cultured from soil and aquatic environments. All Cd adsorption experiments were conducted using 10 ppm Cd and 10 g l^{-1} consortia (wet weight) in a suspension of 0.1 M $NaClO_4$. The consortia were grown using soil broth (SB) or trypticase soy broth (TSB) with 0.5% yeast extract. (■) = Forest soil #1, SB; (□) = wastewater effluent, TSB; (▲) = wastewater effluent, SB; (◇) = forest soil #2, SB; (○) = river water, SB; (●) = wetland water, TSB; (♦) = wetland soil, SB; and (Δ) = wetland water, SB. The dashed curve represents the best-fit adsorption edge that is calculated using the "universal" averaged thermodynamic parameters. Reprinted from Borrok *et al.* (2004a). *Geochim. Cosmochim. Acta* **68**, 3231–3238, with permission from Elsevier.

titration data-sets for individual bacterial species, bacterial consortia, and bacterial cell wall components. They note general similarities between proton adsorption behaviors of the range of bacterial species studied to date, and they use the data to construct an internally consistent surface complexation model for all suitable data-sets, presenting an averaged set of "universal" thermodynamic proton binding and site density parameters for modeling bacterial adsorption reactions in geologic systems. Modeling results demonstrate that the total concentrations of proton-active functional group sites for the 36 bacterial species and consortia tested are remarkably similar, averaging 3.24×10^{-4} moles per wet gram with a 1 σ uncertainty of 1.0×10^{-4} moles per wet gram. Examination of the uncertainties involved in the development of proton-binding modeling parameters suggests that ignoring factors, such as bacterial speciation, ionic strength, temperature, and growth conditions, introduces relatively small error compared to the unavoidable uncertainty associated with the determination of cell abundances in realistic geologic systems. Hence, Borrok *et al.* (2005) proposed that reasonable estimates of the extent of bacterial cell wall deprotonation can be made using averaged thermodynamic modeling parameters, regardless of bacterial species used, ionic strength, temperature, or growth condition of the experiment.

Although a large number of bacterial species appear to exhibit broadly similar adsorption behavior, some studies suggest that at least some bacteria have unique adsorptive properties. For example, Borrok *et al.* (2004b) showed that bacteria that thrive in highly perturbed contaminated environments may exhibit significantly different adsorptive behavior from those from uncontaminated systems. Borrok *et al.* (2004b) measured proton and Cd adsorption onto a range of bacterial consortia grown from heavily contaminated industrial wastes, groundwater, and soils, and modeled the results using a discrete site surface complexation approach to determine binding constants and site densities for each consortium. The results demonstrate that bacterial consortia from different contaminated environments exhibit a range of total site densities (approximately a threefold difference) and Cd-binding constants (approximately a tenfold difference), and Borrok *et al.* (2004b) suggest that the range of adsorption behaviors is related to the evolutionary pressures on the bacteria in each of the contaminated environments.

IV. CONCLUDING REMARKS

The complexity and heterogeneity of realistic natural systems mean that modeling the transport and distribution of mass on a molecular scale is practically impossible due to computation limitations. Conversely, the application of too simple an adsorption model can lead to inaccurate

predictions of metal speciation and mobility in the subsurface (Bethke and Brady, 2000), and therefore models of contaminant transport must include some chemical complexity in order to account for the effects of changing conditions (pH, mineralogy, biomass concentration variability, and so on) on contaminant adsorption behavior. Clearly, a compromise approach must be determined in which sufficient chemical complexity in the form of individual adsorption site specificity for each bacterial and mineral surface is incorporated into geochemical models, but generalities are recognized to enable an approximate solution to be calculated. There is a clear tradeoff between the precision associated with calculated metal speciations and metal distributions and the ability to make such calculations. That is, when modeling metal mobility in the subsurface, generalized models with relatively high degrees of associated uncertainty may be acceptable, while for engineered systems, in which compositional heterogeneities and complexities are well-characterized, more precision is obtainable and a generalized model may be less appropriate than one that explicitly accounts for adsorption site complexities for the particular bacterial species of interest.

This chapter demonstrates that considerable progress has been made both in understanding the molecular-scale mechanisms of metal and proton adsorption onto bacterial cell wall-functional groups and in constructing generalized adsorption models that can be applied to complex systems. However, considerable work remains on both fronts. Mechanistic models of metal adsorption require better characterization of cell wall adsorption sites and adsorption reactions. We must answer questions as fundamental as whether cell wall-functional groups exhibit continuous or discrete deprotonation behavior, we must better constrain the number of types and concentrations of each functional group present on cell walls, and we have relatively poor constraints on the stoichiometries of metal-adsorption reactions. The research summarized in this chapter demonstrates the need to collect mechanistic information from a wide range of experimental approaches in order to rigorously constrain adsorption reaction mechanisms. The most effective approaches used to date include bulk adsorption experiments, a range of spectroscopies, and molecular modeling; however, other approaches are likely to offer new perspectives and should be pursued. Each approach has strengths and weaknesses associated with it, so each provides only a limited view of the overall picture.

In addition to the work required to better characterize metal-adsorption mechanisms on bacterial cell walls, more research is also required in order to understand the effects exerted on metal speciation by biological structures associated with bacteria in natural environments. For example, most bacteria in natural systems do not exist as discrete cells but are present within attached biofilms that are composed primarily of extracellular polymers that may significantly affect metal adsorption and speciation themselves.

While some research has focused on metal binding to these extracellular polymers and to biofilms in general (Acosta *et al.*, 2005; Freire-Nordi *et al.*, 2005; Lau *et al.*, 2005; Marques *et al.*, 1990; Toner *et al.*, 2005), these studies are in their infancy, and considerably more research is required in order to quantify the importance of polymer adsorption relative to cell wall adsorption.

The similar adsorption behavior that is exhibited by most single bacterial species and mixed bacterial consortia, which have been studied to date, suggests that metal-adsorption experiments conducted using only a limited number of types of bacteria (or perhaps even one type) can be extrapolated to reasonably accurately model metal adsorption onto complex mixed bacterial populations if the total concentration of bacteria is known. However, a number of challenges must be addressed before this type of modeling approach can be implemented with confidence. The biggest experimental challenges are to better understand the origin of the observed adsorption similarities and to determine the limits of the universal adsorption-behavior assumption.

Universal adsorption behavior by bacteria would eliminate the need to characterize absolute concentrations and distributions of individual bacterial species in a geologic system. However, the next challenge for application of bacterial-adsorption models to real systems would be to improve our methods for determining overall bacterial site concentrations in realistic settings. While this would be unquestionably more straightforward than mapping out distributions and concentrations of individual bacterial species, quantitative approaches have yet to be designed or tested. It is likely that stochastic-modeling approaches, similar to those used to characterize hydraulic and facies heterogeneities in groundwater aquifers (Huysmans and Dassargues, 2005; Moysey *et al.*, 2003), may prove fruitful.

Mechanistic studies of metal adsorption onto bacterial cell walls and research aimed at field-scale adsorption models are not mutually exclusive activities. The results from each type of study should help to guide the research in the other area. Continued sustained efforts on both fronts eventually will improve not only our molecular-scale understanding of bacterial adsorption reactions but also our ability to apply that understanding to model the effects of adsorption on mass transport in realistic geologic settings.

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