REVIEW OF THE SURFACE CHEMICAL HETEROGENEITY OF BACTERIOGENIC IRON OXIDES: PROTON AND CADMIUM SORPTION

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ABSTRACT. This review focuses on the detailed characterization of proton and metal reactivity on the surface of bacteriogenic iron oxides (BIOS). This substance is composed of a highly porous matrix of ferrihydrite intermixed with intact and fragmented *Gallionella ferruginea* cells. Studies concerning proton and metal cation binding to the BIOS were designed to assess their surface chemical heterogeneity through the further development of two innovative chemical equilibrium models. A multisite Langmuir isotherm model coupled to a linear programming regression method (LPM) was used to assess cadmium (Cd) sorption on bacteriogenic iron oxides. The BIOS proton binding affinity was assessed using a fully optimized continuous pKa spectral model (FOCUS). LPM analysis of the results from BIOS potentiometric experiments suggested the presence of two Cd binding sites on the whole BIOS, and three on the organic (bacteria) fraction surface. LPM results were able to show the non-additive nature of the BIOS mixture. The proton speciation characteristics of the BIOS surface, and that of the organic (bacteria) fraction were probed using FOCUS. This analysis revealed a striking similarity between the surface reactivity of the BIOS mixture and that of pure iron oxyhydroxide phases such as goethite and lepidocrocite. This, in turn, was a clear indication of the extensive masking of underlying surface bacterial (organic) functional groups by ferrihydrite precipitate. FOCUS results emphasized the non-additive nature of the BIOS composite. This was further corroborated by the results for Cd^{2+} sorption experiments obtained from LPM analysis.

INTRODUCTION

Living organisms depend strongly on surface and underground freshwater sources for their subsistence. Through the course of millions of years of Earth's history, these waters have acquired a well-defined chemical composition suitable for life (Honeyman and Santschi, 1988; Stumm and Morgan, 1996). Addition of trace amounts of foreign organic and inorganic species to these waters alters their pristine composition. These alterations make them unsafe for consumption by living organisms (Singer and others, 2000; Puig-Grajales and others, 2000). Toxicity in aqueous environments stems, in part, from an artificial influx of toxic metals generated by human industrial activity (Honeyman and Santschi, 1988; Stumm and Morgan, 1996; Singer and others, 2000; Puig-Grajales and others, 2000). Species of Cd, Pb, Cu, Zn, and As among others, have been detected in aquatic ecosystems and found to bioaccumulate in organisms in nanomolar to micromolar concentrations (Honeyman and Santschi, 1988; Warren, and others, 1998; Takács and others, 1999; Pandey and others, 2000).

Cadmium has been reported to be among the most toxic contaminant metals present in freshwater systems (Waisberg and others, 2003). Cadmium has been listed by the United States Environmental Protection Agency (EPA) as one of 126 priority pollutants, with a half-life in humans of 15 to 20 years (Waisberg and others, 2003). Hare and Tessier (1996), showed that the effective free Cd^{2+} concentration in a living organism is modulated by both the ability of the metal to complex to dissolved organic

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matter, and by the competition of free Cd^{2+} and H^+ for biological uptake sites. In addition, the free Cd^{2+} concentration available for uptake by a living organism may be reduced by the ability of geochemically reactive substances to complex toxic metal cations (Honeyman and Santschi, 1988; Warren and others, 1998; Takács and others, 1999; Fowle and Fein, 1999; Pandey and others, 2000; Daughney and others, 2001; Châtellier and others, 2001; Phoenix and others, 2002; Yee and Fein, 2001, 2003).

In order to determine the fate of toxic metal species in aquatic environments and assess their impact on living organisms, scientific disciplines, such as environmental chemistry, physiology and toxicology have, in the mid-1990s, collaborated to shape what is now known as the Biotic Ligand Model (BLM). This new chemical equilibrium approach attempts to calculate and combine parameters for the partitioning of toxic metal cations, not only on traditional components such as dissolved organic matter, but simultaneously within living physiological systems. The Biotic Ligand Model (BLM) envisions an improved understanding of the underlying chemical processes, which determine the aqueous form of a toxic metal available to an organism. Most importantly, the model provides a link between toxic metal bioavailability to an organism and its response to that particular toxin (Paquin and others, 2002).

Natural organic ligands, dissolved organic matter, bacterial cells, mineral oxide colloids, and bacteriogenic iron oxides are ubiquitous in natural freshwater systems (Stumm and Morgan, 1996; Lovley, 2000). Metal partitioning parameters on these substances can be approximated using innovative modeling approaches and high quality metal sorption data (Fein and others, 1997; Martinez and Ferris, 2001; Smith and Ferris, 2001). In the realm of the Biotic Ligand Model (BLM), these metal speciation calculations are of outmost importance, since they would be essential for the control and monitoring of dissolved toxic metal forms in aqueous systems. This would enable researchers to predict and possibly minimize the amount of a toxic metal species reaching vulnerable biotic ligands (living organisms), as suggested previously by Paquin and others (2002).

As part of the ongoing effort to provide remediation solutions to environmental problems, several studies have emphasized the importance of investigating the behavior of bacteria and bacteriogenic iron oxide surfaces as geochemically reactive solids in aquatic ecosystems. Toxic metal sorption mechanisms onto these types of particulate matter have gained considerable attention because of their inherent ability to remove or at least control the speciation of metals in contaminated freshwater systems. These studies have been concerned with understanding the mechanisms by which cations such as Pb^{2+} , Cd^{2+} , Ni^{2+} , Zn^{2+} and Cu^{2+} bind to acidic and non-acidic functional groups on bacterial cell and bacteriogenic iron oxide surfaces (Plette and others, 1995; Fein and others, 1997; Seki and others, 1998; Zhou and others, 1998; Puranik and Paknikar, 1999; Sanchez and others, 1999; Ferris and others, 1999; Fowle and Fein, 1999; Qiming and Pairat, 2000; Small and others, 2001; Daughney and others, 2001; Yee and Fein, 2001, 2003; Martinez and others, 2004).

This review will focus on two innovative chemical equilibrium modeling approaches recently applied to the quantification of proton and metal binding on the surface of composite bacterial cell-iron oxide mixtures (Martinez and others, 2003, 2004), defined through out this review as bacteriogenic iron oxides (BIOS). Specifically, continuous (Smith and Ferris, 2001; Martinez and others, 2003) and discrete (Brassard and others, 1990; Smith and Kramer, 1999; Martinez and Ferris, 2001; Martinez, Pedersen and Ferris, 2004) affinity pK spectrum methods were applied to analyze proton and Cd^{2+} complexation to the reactive surface of the BIOS mixture and compare it to that of its end member constituents, namely, intact and fragmented bacterial cells embedded within 2-line ferrihydrite precipitates.

Fig. 1. SEM picture of BIOS mixture. Label **A** indicates the *Gallionella ferruginea* stalks. **B** depicts the iron oxyhydroxide precipitates on the bacterial stalks. The picture clearly shows the BIOS mixture as a complicated matrix of intact and fragmented bacterial embedded within a 2-line ferrihydrite precipitate.

BACTERIA AND BACTERIOGENIC IRON OXIDE SURFACE HETEROGENEITY

Bacteriogenic iron oxides (BIOS), abundant in surface water and groundwater systems, are potent sorbents of dissolved metal cations (Warren and Ferris, 1998; Ferris and others, 1999, 2000; Lovley, 2000). This property emphasizes their importance in regulating the dispersion of toxic metal species in pristine and contaminated environments. As mentioned previously, BIOS are complex materials composed of poorly ordered iron oxyhydroxide precipitates (that is, 2-line ferrihydrite) intermixed with intact and partly degraded bacterial cells, as shown by SEM analysis in figure 1 (Warren and Ferris, 1998; Ferris and others, 1999, 2000; Lovley, 2000). BIOS are formed in anoxic/oxic water mixing zones, at low pO_2 , high Fe^{2+} concentration and circumneutral pH by the metabolic activity of the stalk forming bacteria, *Gallionella ferruginea* and other iron oxidizing microorganisms (Warren and Ferris, 1998; Ferris and others, 1999, 2000; Lovley, 2000). The BIOS metal binding potential is a function of the type, concentration and availability of iron oxyhydroxide and bacterial cell surface functional groups, and their respective acidity constants (K_a). Similarly, the BIOS metal

binding strength is a function of the type, concentration and interaction of iron oxyhydroxide and bacteria surface functional groups. It can be quantified using an apparent equilibrium constant K_s as defined previously (Martinez and others, 2004). Bacterial cell wall types can be identified according to a light microscopy staining procedure known as the Gram stain; Gram+ cells stain blue, where as Gram- ones stain red (Beveridge, 1981; Madigan and others, 2000). The Gram+ bacterial cell wall, such as that of *Bacillus subtilis*, consists primarily of peptidoglycan (90%) and smaller amounts of teichoic acids. Teichoic acid is a general group of compounds, which consists of glycerophosphate or ribitol phosphate residues. These polyalcohols are connected by phosphate esters and usually have other sugars and D-alanine attached (Beveridge, 1981; Madigan and others, 2000). Teichoic acids are, in general, negatively charged at neutral pH and in consequence they can regulate the passage of positively charged metal ions through the cell membrane. Some teichoic acids in the Gram+ cell wall are associated with lipids. These are called lipoteichoic acids. Within the complex array of the Gram+ surface structure, the same basic functional groups, contribute to the sorption of toxic metal cations (Madigan and others, 2000).

The Gram- cell wall, such as that of *Escherichia coli*, is a multilayered structure that also contains peptidoglycan; however an outer lipopolysaccharide (LPS) layer is also present. This layer usually denoted as outer membrane is essentially a lipid bilayer, which contains LPS, phospholipids and protein. The LPS of the Gram- surface consist of two portions; (a) core-polysaccharides and (b) O-polysaccharides (Madigan and others, 2000). The core consists of ketodeoxyoctonate (KDO) and sugar subunits such as glucose and galactose, as well as heptoses. The O-polysaccharide contains a wide variety of sugars including rhamnose and mannose subunits (Beveridge, 1981; Madigan and others, 2000). Sugars have hydroxyl functional groups, which do not readily interact with metal cations until high pH values are reached, and have net neutral charge below a pH of approximately 10.

Spectroscopic studies have shown that the iron oxyhydroxide phase in the BIOS mixture consists primarily of poorly ordered 2-line ferrihydrite. The crystal structure of this oxyhydroxide has not yet been well resolved mainly because of the absence of well-defined maxima in XRD spectra (Jambor and Dutrizac, 1998). However, EXAFS studies indicate that the Fe-(O,OH) bond lengths in ferrihydrite are characteristic of Fe^{3+} octahedral coordination as in goethite (Jambor and Dutrizac, 1998). Other works suggest that 25 percent of the Fe^{3+} in ferrihydrite is in tetrahedral coordination at the surface, while the bulk iron has an arrangement similar to that of octahedral oxyhydroxides (Jambor and Dutrizac, 1998). These spectroscopic results may suggest that a resemblance might exist between the surface oxygen atom coordinations on goethite and those on 2-line ferrihydrite. These oxygen atom arrangements have been assigned IUPAC designations, where oxo (hydroxo), μ -oxo (hydroxo) and μ_3 -oxo (hydroxo) refer to an oxide (hydroxide) ion bound to one $\text{FeO}(\text{H})$, two $\text{Fe}_2\text{O}(\text{H})$, and three $\text{Fe}_3\text{O}(\text{H})$, Fe atoms respectively (Rustad and others, 1996; Hiemstra and Van Riemsdijk, 1996; Hiemstra and others, 1996). MUSIC/CD-MUSIC models and molecular static (quantum mechanical) calculations propose that each of these configurations has a characteristic acidity constant (K_a), which depends also on the mineral face being considered (Rustad and others, 1996; Hiemstra and Van Riemsdijk, 1996; Hiemstra and others, 1996; Felmy and Rustad, 1998).

The aforementioned description of bacterial cell wall chemical structure, along with the results of XRD spectroscopy clearly emphasize the structural complexity of the BIOS, as inferred from figure 1. This degree of complexity should in turn suggest that the particulars of BIOS end-member component reactive behavior, such as additivity, be considered for a complete assessment of surface metal sorption mechanisms (Westall and others, 1995; Vermeer and others, 1999). The additivity rule, as defined

by Vermeer and others (1999), states that a reactive solid would be considered additive, when the sum of its individual end-member metal sorption capacities equal that of the composite (Vermeer and others, 1999). If the binding capacity of the composite differs from those of its end-members, then this would constitute a deviation from the additivity rule and may further imply specific chemical interactions between end-member solid phase functional groups (Vermeer and others, 1999). Vermeer and others (1999) suggested that the overall adsorption of a metal ion to a complex such as the BIOS will be smaller than predicted by the additivity rule when the metal ion shows a greater affinity for the organic rather than the iron oxide fraction (Vermeer and others, 1999).

Furthermore, as suggested by figure 1, the complexity of intact bacterial cell surfaces and bacteriogenic iron oxide mixtures has been the limiting factor in the efficacy of chemical equilibrium and electrostatic models to describe proton and metal reactivity on these surfaces (Westall and others, 1995). These models, as stated previously, have been mainly applied to the study of mineral surfaces and simpler organic ligand reactivity. Under these conditions, one or at most two binding sites are needed to envision proton and metal surface functional group interactions. Complex structures, with many reactive sites in a number of chemical microenvironments, such as bacteriogenic iron oxides and humic substances, need caution in the application of electrostatic models and at least an *a priori* refinement of chemical equilibrium models, attempts of which are described in the following sections.

CHEMICAL EQUILIBRIUM MODELING TECHNIQUES

In order to explore the reactive chemical heterogeneity of bacteriogenic iron oxides and their end-member components, two chemical equilibrium models, described below, were developed. The essential principles of these models are explained in detail in Martinez and Ferris (2001), and Martinez and others (2002), for the investigation of bacterial cell surface heterogeneity, and Martinez and others (2003), and Martinez, Pedersen and Ferris (2004), for the analysis of bacteriogenic iron oxide potentiometric data.

Multisite Langmuir Approach and the Linear Programming Model

High resolution Cd^{2+} -BIOS or bacteria, B, complexation data can be modeled in using linear programming (LPM), as described previously by Martinez and others (2004). In order to describe the interaction of metal cations and protons with the BIOS or bacterial phase reactive surfaces, a $\text{Cd}^{2+}/\text{H}^{+}$ competition reaction is assumed to take place in a 1:1 ratio, as follows:

where, B_j represents a surface reactive site and $K_{S,j}$ is the concentration apparent equilibrium constant for the reaction in equation (1) above, conditional on ionic strength. For a j th deprotonated binding site at the i th step of the titration, $K_{S,j}$ can be defined as:

$$K_{S,j} = \frac{[\text{CdB}_j^{+}]_i \cdot [\text{H}^{+}]_{\text{meas},i}}{[\text{Cd}^{2+}]_{\text{meas},i} \cdot [\text{B}_j\text{H}^0]_i} \quad (2)$$

for $i = 1 \dots n$ titrant additions and $j = 1 \dots m$ binding sites. In the above expression, $K_{S,j}$ implicitly embodies electrostatic parameters and is a function of experimentally determined proton and metal concentrations, ($[\text{H}^{+}]_{\text{meas},i}$ and $[\text{Cd}^{2+}]_{\text{meas},i}$) and of the amount of Cd^{2+} bound to the j th site at the i th step of the titration, $[\text{CdB}_j^{+}]_i$. From

mass balance principles, the total bound metal at the i th titrant addition, $[\text{CdB}^+]_{\text{T},i}$, and the total ligand concentration, $[\text{B}]_{\text{T}}$ can be expressed as:

$$[\text{CdB}^+]_{\text{T},i} = \sum_{j=1}^m [\text{CdB}_j^+]_i = [\text{Cd}^{2+}]_{\text{T}} - [\text{Cd}^{2+}]_{\text{meas},i} \quad (3)$$

and

$$[\text{B}]_{\text{T}} = \sum_{j=1}^m [\text{B}_j] = [\text{CdB}^+]_{\text{T},i} + \sum_{j=1}^m [\text{B}_j\text{H}^0]_i \quad (4)$$

where $[\text{B}_j]$ refers to the individual site density for a particular surface functional group type. The total concentration of bound metal, $[\text{CdB}^+]_{\text{T},i}$, can be expressed as a sum of complexed metal concentrations for each of the j th surface ligands at the i th step of the titration ($[\text{CdB}^+]_{\text{T},i} = \sum_{j=1}^m [\text{CdB}_j^+]_i$). However, experimental measurements of total, $[\text{Cd}^{2+}]_{\text{T}}$ and free metal concentrations $[\text{Cd}^{2+}]_{\text{meas},i}$, only allow direct determination of $[\text{CdB}^+]_{\text{T},i}$, as indicated by equation (3). Equation (4) above assumes negligible concentrations of surface deprotonated functional groups (B^-). Therefore, although caution should be exercised when interpreting these results in terms of chemical equilibrium mass balance, it should also be noted that a high concentration of metal ($[\text{Cd}^{2+}] = 100 \mu\text{M}$) was required for the BIOS experiments mentioned in this review. This could suggest a negligible concentration of unbound, deprotonated surface ligands, which should justify the simplification applied in equation (4).

The fraction of the total j th ligand concentration, bound by the metal cation at the i th step of the titration, $\alpha_{\text{ML},ij}$, can be expressed as a function of the bound metal at the i th titrant addition, $[\text{CdB}_j^+]_i$ and the j th ligand concentration, $[\text{B}_j]$ as follows:

$$\alpha_{\text{ML},ij} = \frac{[\text{CdB}_j^+]_i}{[\text{B}_j]} = \frac{[\text{CdB}_j^+]_i}{[\text{CdB}_j^+]_i + [\text{B}_j\text{H}^0]_i} \quad (5)$$

The protonated j th ligand concentration at the i th step of the titration, $[\text{B}_j\text{H}^0]_i$ can be in turn expressed as a function of $[\text{CdB}_j^+]_i$, by rearranging the expression for the equilibrium constant $K_{\text{S},j}$ in equation (2). The calculated bound metal concentration at the i th titrant addition, $[\text{CdB}^+]_{\text{T},\text{calc},i}$, can then be determined as a function of measured, ($[\text{H}^+]_{\text{meas},i}$ and $[\text{Cd}^{2+}]_{\text{meas},i}$) and adjustable ($[\text{B}_j]$) parameters, as shown below:

$$[\text{CdB}^+]_{\text{T},\text{calc},i} = \sum_{j=1}^m (\alpha_{\text{ML},ij} \cdot [\text{B}_j]) = \sum_{j=1}^m \left(\frac{[\text{Cd}^{2+}]_{\text{meas},i} \cdot K_{\text{S},j}}{[\text{Cd}^{2+}]_{\text{meas},i} \cdot K_{\text{S},j} + [\text{H}^+]_{\text{meas},i}} \cdot [\text{B}_j] \right) \quad (6)$$

The linear programming approach to solving chemical equilibrium problems for multi-site metal sorption is based on solving a matrix equation $\bar{b} = \text{A} \cdot \bar{x}$ for $\bar{x}$. Here A is an $n \times m$ matrix of $\alpha_{\text{ML},ij}$ entries as defined in equations (5) and (6). $\bar{b}$ is a $n \times 1$ vector of calculated bound metal concentrations for each titrant addition, $[\text{CdB}^+]_{\text{T},\text{calc},i}$, as defined in equation (6). The $m \times 1$ vector $\bar{x}$ contains the adjustable parameters, $[\text{B}_j]$, for each of the m binding sites.

Linear programming regression techniques minimizes the number of binding sites and the absolute error, $e = |[\text{CdB}^+]_{\text{T},\text{calc},i} - [\text{CdB}^+]_{\text{T},i}|$, rather than the least squares using a simplex search method (Brassard and others, 1990). This approach finds one global minimum for the error function, which emphasizes zero as a possible solution and avoids convergence problems such as those found in FITEQL where the

solution could be a local minimum (Brassard and others, 1990; Smith and Kramer, 1999; Martinez and Ferris, 2001). As stated earlier, the linear programming approach uses a grid of fixed $pK_{s,j}$ values and optimizes parameters such as total binding site concentrations. Each site density, $[B_j]$, is assigned a positive value where zero is a possible result. This generates a $pK_{s,j}$ spectrum where discrete metal binding sites are determined by the number of $pK_{s,j}$ values, which have a corresponding nonzero metal binding site density. When $[B_j]$ values are added, their sum should approximate the total available ligand concentration on the sorbent surface, $[B]_T$, for a maximum experimental pH value (Brassard and others, 1990; Smith and Kramer, 1999; Martinez and Ferris, 2001; Martinez and others, 2004).

Proton Binding and the Fully Optimized Continuous (FOCUS) pK_a Spectrum Approach

Acid base titration data from the BIOS composite and bacterial cell fractions were analyzed using MATLAB 6.0 (The Mathworks Inc. MA). Proton dissociation mechanisms for a single protonated site were modeled using the reaction below:

where, BH refers to a protonated surface binding site, $H^+ \sim H_3O^+$ is the hydronium ion species, whose activity, $\{H^+\}$, was measured with a pH electrode. $\{H^+\}$ was converted to concentration through the relationship $\{H^+\} = \gamma_H \cdot [H^+]$, where γ_H is the tabulated value for the proton activity coefficient, as in Harris, 1995, at the corresponding ionic strength. B^- describes a deprotonated reactive surface functional group with a net negative charge. Finally, K_a is the apparent proton dissociation constant for BH in equation (7), conditional on ionic strength, and described as follows:

$$K_a = \frac{[B^-] \cdot [H^+]}{[BH]} \quad (8)$$

where, $pK_a = -\log_{10} K_a$ is a measure of functional group acid strength as described previously.

In order to make acid base titration data useful for the study of proton dissociation constants, the raw data obtained from measuring the solution's pH as a function of titrant added, must be transformed to remove the effects of the dissociation of water which dominate the shape of the raw titration curve (Martinez and others, 2002). Once this is done, the mechanisms of deprotonation and protonation reactions on the substrate of interest can be resolved (Smith and Ferris, 2001; Martinez and others, 2002). The usual transformation of the raw acid base titration data is shown below for the i th addition of titrant:

$$b_{\text{meas},i} = Cb_i - Ca_i + [H^+]_{\text{bulk},i} - [OH^-]_{\text{bulk},i} \quad (9)$$

where $b_{\text{meas},i}$ represents the experimental net surface charge excess. Ca_i and Cb_i correspond to the acid and base concentrations respectively at the i th addition of titrant and $[H^+]_{\text{bulk},i}$ and $[OH^-]_{\text{bulk},i}$ are calculated from pH measurements. The bulk hydroxide ion concentration, $[OH^-]_{\text{bulk},i}$, was calculated using the relationship, $[OH^-]_{\text{bulk},i} = K_w / [H^+]_{\text{bulk},i}$, where $[H^+]_{\text{bulk},i} = \{H^+\} / \gamma_H$ and $K_w = 1.01 \times 10^{-14}$ at 25°C (Harris, 1995). The value of the proton activity coefficient ($\gamma_H = 0.83$) corresponded to an ionic strength of 0.1 M and a temperature of 25°C (Harris, 1995).

As described previously (Brassard and others, 1990; Martinez and others, 2002), the charge excess, b , can be calculated as a function of measured ($[H^+]_{\text{bulk}}$) and adjustable ($[B_j]$) speciation parameters as follows:

$$b_{\text{calc},i} = \sum_{j=1}^m \left(\frac{K_{aj}^{\text{app}}}{K_{aj}^{\text{app}} + [\text{H}^+]_{\text{bulk},i}} \right) \cdot [\text{B}_j] + S_o \quad (10)$$

where m refers to the number of binding sites, $[\text{B}_j]$ is the site density in $\mu\text{moles/mg}$ of sorbent, and K_{aj}^{app} is the apparent acidity constant for the j th site as per equation (8). The constant offset term, S_o , is necessary in order to account for positive charges on the surface (Brassard and others, 1990; Martinez and others, 2002). For a true monoprotic system, S_o corresponds to the difference in concentration between the sites that are always protonated and those that are always deprotonated at the i th step of the titration (Martinez and others, 2002). It is important to note that the resolution of acid base titration experiments may not be sufficient to detect changes in charge contributions from specific chemical structures from within the reactive solid surface microenvironments. Therefore, equation (10) above, assumes a monoprotic system from which pK_a and site density values, $[\text{B}_j]$, are determined under the assumption that each functional group type in the reactive surface has a Gaussian pK_a distribution (Smith and Ferris, 2001; Martinez and others, 2002). In addition, the total binding site density $[\text{B}]_T$, is calculated as the sum of the individual site concentrations for each site type, $[\text{B}]_T = \sum_{j=1}^m [\text{B}_j]$.

Numerical difficulties exist when attempting to fit the mathematical models in equations (6) and (10), to the experimental data described by equations (3) and (9) respectively, because binding constants and site densities are correlated parameters. In both the discrete and continuous binding site analysis models presented herein and explained in detail by Brassard and others (1990), and Smith and Ferris (2001) respectively, the correlation problem was solved by fixing the values of the dissociation constants on a fixed interval grid and writing the problem in matrix form for n titration points and m binding sites as $\bar{b} = A \cdot \bar{x} + S_o$, where A is an $n \times m$ matrix of $\alpha_{(i,j)}$ terms, $\bar{x}$ is a $m \times 1$ vector of binding site concentrations, and $\bar{b}$ is a $n \times 1$ vector containing the measured charge excess from the titration data, as presented in equation (9). The $\alpha_{(i,j)}$ terms in matrix A can be defined as shown below:

$$\alpha_{(i,j)} = \frac{K_{aj}^{\text{app}}}{K_{aj}^{\text{app}} + [\text{H}^+]_{\text{bulk},i}}, \quad \text{for } i = 1 \dots n \text{ and } j = 1 \dots m \quad (11)$$

The nature of the matrices as described above makes this an *ill-posed* problem. This means that small changes in S_o lead to large changes in $\bar{x}$ and that more than one error minimum can be found from optimization for $\bar{x}$ as a solution to the equation $\bar{b} = A \cdot \bar{x} + S_o$, unless additional assumptions are made about the nature of the numerical solution (Martinez and others, 2002).

A regularization approach to solving *ill-posed* problems has been recently applied to acid base data (Smith and Ferris, 2001; Martinez and others, 2002). The regularization process involves *a priori* assumptions about the system to constrain the results. This leads to less sensitivity to data changes and a unique solution (Martinez and others, 2002). The results can be regularized for a few discrete sites or for smoothness depending on whether a discrete or continuous affinity distribution result is needed. This is an *a priori* assumption about the final result, which allows the formulation of the regularized least squares optimization problem. The FOCUS calculations can be summarized as follows:

$$\begin{aligned}
 &\text{minimize } SS + \lambda R \\
 &\text{where } SS = \sum_{i=1}^n (b_{\text{calc},i}(x) - b_{\text{meas},i})^2 \\
 &\text{and } R(x_1, \dots, x_m) = \sum_{j=2}^{m-1} (x_{j-1} - 2x_j + x_{j+1})^2 \\
 &\text{such that } x_j \geq 0 \text{ for } j = 1 \dots m
 \end{aligned} \tag{12}$$

where the SS term is the sum of squares term for nonlinear regression, and R represents the regularization term. Both SS and R are dependent on the parameter vector $\bar{x}$ and the regularization power is controlled by the constant λ . To determine the optimal solution an initial fit is performed ignoring the regularization term such that $\lambda R = 0$, where $\lambda = 0$, and the minimum SS value ($SS_{\min}$) is then determined. The optimal λ , in FOCUS, is found by minimizing the distance, d , between two points in $(SS, \lambda R)$ - space, defined by:

$$d(\lambda_K) = \sqrt{(SS_{\min} - SS_K)^2 + (0 - \lambda_K R_K)^2} \tag{13}$$

The distance d is minimized, by iterating through k values of λ . At the k^{th} value of λ , SS_K and R_K are determined and $d(\lambda_K)$ is a minimum. The mechanism of finding the best value of λ is what defines the FOCUS method as being fully optimized. A detailed account of optimization methods and routines used for the FOCUS method can be found in Smith and Ferris (2001).

BIOS PROTON SORPTION PROPERTIES

In Martinez and others (2003), the FOCUS method, described earlier, was used to optimize transformed acid base titration data for the BIOS and their organic fraction. This analysis generated surface charge excess curves which suggested apparent point of zero charge values (pH_{pzc}) of 9.6 ± 0.1 and 4.1 ± 0.1 for the BIOS and bacterial fractions, respectively. Points of zero charge (pH_{pzc}) were estimated at the pH where the surface charge excess was closest to 0. These estimates should not be regarded as fully equivalent to actual points of zero charge, which are conventionally determined from acid base titration experiments at different ionic strengths (Smith and Ferris, 2001; Martinez and others, 2002).

The apparent pH_{pzc} value of 4.1 ± 0.1 determined for the bacterial cell fraction was directly comparable to the value of 4.5 ± 0.1 reported earlier for Gram-positive bacterial cell wall fragments (Plette and others, 1995). This agreement implied a similar charging behavior between bacteria grown in pure culture and the organic fraction of the BIOS. This clearly emphasized the biogenic origin of the hydroxyl-amine insoluble fraction. For the BIOS composite, the apparent pH_{pzc} of 9.6 ± 0.1 was consistent with values of 9.4 and 8.6 to 9.3 reported for goethite and hematite, respectively (Hiemstra and others, 1996; Felmy and Rustad, 1998). Although a detailed characterization of the 2-line ferrihydrite structure and direct evidence of electrostatic charging properties are needed, the apparent pH_{pzc} of 9.6 ± 0.1 , and evidence of Fe octahedral coordination (Felmy and Rustad, 1998), inferred a correlation between the crystalline structure and surface charging behavior of goethite and the BIOS' 2-line ferrihydrite. The pH_{pzc} of 9.6 ± 0.1 suggests further that the BIOS mixture surface charge is dominated by contributions from reactive groups of ferrihydrite rather than those of bacterial origin. The implication is that acidic (that is, low pK_a) groups associated with and contributing to the low apparent pH_{pzc} of the bacterial cell fraction

TABLE 1
Summary of pK_a values for each site^a

Sample	Site 1	Site 2	Site 3	Site 4
BIOS	4.27 ± 0.51	6.61 ± 0.55	7.89 ± 0.88	9.65 ± 0.66
Bacteria	4.18 ± 0.37	4.80 ± 0.54	6.98 ± 0.45	9.75 ± 0.68
Residual	4.10 ± 0.43	6.53 ± 0.45	7.81 ± 0.76	9.51 ± 0.68

^aFor each site the column entries correspond to the pK_a value plus or minus the width ($\pm 2\sigma$) of the mean pK_a value determined from a Gaussian model for the peak shapes.

interact chemically with ferrihydrite, and thus are not subject to titration in composite BIOS samples (Ferris and others, 2000; Small and others, 2001).

FOCUS optimization provided pK_a spectra for the BIOS and bacterial cell fractions, respectively, upon minimization of the error between the measured and calculated charge excess quantities. Through a residual deconvolution approach, a continuous affinity spectrum was generated to portray the concentration and pK_a distribution of proton binding sites present on the 2-line ferrihydrite portion of the BIOS. Deconvolution of the BIOS spectrum intended to remove overlapping site density contributions from the bacterial cell surface functional groups. The deconvoluted pK_a spectrum, corresponding to the pure iron-oxyhydroxide phase, was calculated using the weighted mass fraction of the hydroxylamine insoluble phase, as recorded in Martinez and others (2003). This type of plot was generated to show the distribution of proton binding sites present on the 2-line ferrihydrite portion of the BIOS sample (positive values), as well as sites lost in the composite (negative values) owing to surface chemical interactions between the bacteria and 2-line ferrihydrite functional groups.

The apparent pK_a results for the BIOS in Martinez and others (2003), summarized in table 1, were compared to values of molecular modeling studies reported previously (Hiemstra and others, 1996; Felmy and Rustad, 1998). The value of 4.27 ± 0.51 was in good agreement with acidity constants of 3.80 and 4.00, and 4.30 for lepidocrocite and the μ_3 -hydroxo coordination on the (110) goethite face respectively, as calculated by the MUSIC model (14). Values of 7.89 ± 0.88 and 9.65 ± 0.66 were consistent with those reported for the aquo (110) and μ -hydroxo (021) coordination with pK_a values of 8.05 and 9.79, respectively. The experimental pK_a of 7.89 ± 0.88 was also comparable to other acidity constants calculated for μ_3 -hydroxo in the (110) goethite face ranging from 8.61 to 8.84 (Rustad and others, 1996; Felmy and Rustad, 1998). These observations are all consistent with the proposed octahedral arrangement of the Fe atoms in ferrihydrite, as mentioned in Jambor and Dutrizac (1998). The value of 6.61 ± 0.55 in table 1 for the BIOS may be attributable to the μ -hydroxo conformation on the (110) face of goethite, as suggested by Rustad and others (1996).

The BIOS mixture was then treated with a potent reducing agent, hydroxylamine hydrochloride ($\text{NH}_2\text{OH} \cdot \text{HCl}$), to remove the iron oxide phase. The results from ICP-OES analysis showed that 95.1 percent of the iron originally present in the BIOS (6.55 ± 0.09 mg/L), was removed in the bacteria fraction by $\text{NH}_2\text{OH} \cdot \text{HCl}$ treatment. As reported by Dong and others (2003), processing of the BIOS with hydroxylamine hydrochloride ($\text{NH}_2\text{OH} \cdot \text{HCl}$) avoids substantial damage to the cells of microorganisms as inferred from microscopic examination (Dong and others, 2003). This process proved to be suitable for the preparation of a representative sample of intact and fragmented *Gallionella ferruginea* cells for subsequent potentiometric experiments, as shown by the results in Martinez and others (2003) and Martinez, Pedersen and Ferris (2004).

The FOCUS result for the bacterial cell fraction acid-base transformed data generated acidity constants of 4.18 ± 0.37 , 4.80 ± 0.54 , 6.98 ± 0.45 and 9.75 ± 0.68 (table 1). These values were found to be consistent with the ranges of 2 to 6, 5.6 to 7.2 and 9 to 11 for carboxyl, phosphate and amine functional groups reported for intact and fragmented bacterial cells (Fein and others, 1997; Cox and others, 1999; Sokolov and others, 2001; Martinez and others, 2002). These equilibrium constants differed from those of the BIOS mixture and were in good agreement with previously reported bacterial surface acidity constants (Fein and others, 1997; Cox and others, 1999; Sokolov and others, 2001; Martinez and others, 2002). This observation implicated masking of cell surface reactive groups, which were not detected through acid base titration methods due to the interaction of iron oxide (2-line ferrihydrite) with the cell surface (Vermeer and others, 1999; Small and others, 2001).

The deconvoluted pK_a spectrum for the pure iron-oxyhydroxide phase (2-line ferrihydrite), calculated as mentioned earlier, was found to be in good agreement with the discrete MUSIC pK_a spectra for goethite and lepidocrocite (Dzombak and Morel, 1990). A pK_a value of 6.53 ± 0.45 , (table 1), for the residual spectrum, coincides with those at 6.2 and 6.4 in a lepidocrocite MUSIC spectrum (Venema and others, 1998). This emphasized the non-organic nature of the pK_a at 6.61 ± 0.55 found in the BIOS deconvoluted spectrum. pK_a values of 7.89 ± 0.88 and 7.81 ± 0.76 , for the BIOS and ferrihydrite fractions respectively, were shown to be consistent with the goethite discrete spectra and were not observed for the bacterial fraction. This suggested specific bacterial functional group masking by iron oxyhydroxide interactions. The potential resemblance of the surface reactivity of 2-line ferrihydrite with more stable iron oxide phases, such as goethite and lepidocrocite, could have an effect on the long term stability and mobilization of metal cations within the BIOS matrix.

Comparison of the pK_a values calculated using FOCUS (table 1), in Martinez and others (2003), and those from molecular static calculations (Rustad and others, 1996; Felmy and Rustad, 1998), emphasized the use of continuous pK_a spectra as a means to determine acidity constants of natural iron oxide surfaces. However, because the structure of 2-line ferrihydrite is not well resolved (Jambor and Dutrizac, 1998), and due to the uncertainty that persists in iron oxide acid dissociation constant calculations (Santelli and others, 2001), only a qualitative comparison of pK_a values was feasible. Nonetheless, this was an important step forward in trying to assess the surface heterogeneity of BIOS through the use of thermodynamic equilibrium models.

Detailed electrostatic calculations on the BIOS surface should not be possible using the present form of current surface complexation models because of the complicated, structural nature of the BIOS mixture. It should be emphasized that direct spectroscopic methods, such as extended X-ray absorption fine structure (EXAFS) and FTIR should, respectively, be able to provide a better understanding of the proton and metal interactions within the BIOS mixture, under different background electrolyte concentrations and other environmental conditions.

Individual $[B_j]$ and total $[B]_T$ site densities for the BIOS phases, summarized in table 2, were determined by FOCUS optimization of acid base titration data. Binding site concentrations of 4.20, 1.26 and 3.00 $\mu\text{moles/mg}$ BIOS were found to be consistent with those reported separately for bacteria and iron oxides (Smith and Ferris, 2001; Martinez and others, 2002). Total and individual site densities were normalized to $\mu\text{moles/mg}$ of BIOS using mass fractions of 0.345 and 0.655 for bacteria and ferrihydrite respectively, as depicted in Martinez and others (2003).

The interpretation of the above parameters, as depicted in Martinez and others, 2003, attests to the complicated structural nature of the BIOS composite. Such an arrangement, composed of intact and fragmented bacteria embedded within a poorly ordered iron oxide phase, is bound to have uncertainties in size, geometry and

TABLE 2
Summary of total and individual binding site density (L_T) values^a

Sample	B ₁	B ₂	B ₃	B ₄	Total B _T
BIOS—μmoles/mg BIOS	0.95	0.15	1.25	1.85	4.20
Bacteria—μmoles/mg BIOS	0.09	0.38	0.07	0.72	1.26
Residual—μmoles/mg BIOS	0.55	0.20	0.85	1.40	3.00

^aSite densities are in units of μmol/mg of BIOS, normalized as explained in text. These were calculated using the area under Gaussian curves, as described in Martinez and others, 2003. The B₁ to B₄ labels correspond to sites 1 to 4 in table 1. The total B_T column is the sum of the individual B values for sites 1 to 4.

penetrability by counter ions, as suggested for humic colloids (Westall and others, 1995). These characteristics would limit the ability of electrostatic models (CD-MUSIC and the classic 2-pK_a methods) to predict intrinsic speciation chemistry (Stumm and Morgan, 1996; Rustad and others, 1999; Boily and others, 2001). An empirical model such as FOCUS, which implicitly embodies adjustable electrostatic parameters, would provide a more realistic approximation of the BIOS surface chemistry (Stumm and Morgan, 1996; Rustad and others, 1999).

The surface chemical heterogeneity of the BIOS composite and its end-member components was recovered using FOCUS pK_a spectroscopy. The analysis of pK_a spectra and binding site concentrations in the BIOS and its organic and mineral fractions, indicated masking of bacterial surface functional groups by iron oxyhydroxides. The reactive surface heterogeneity of the BIOS was inferred to be analogous to that of pure iron oxide phases of ferrihydrite and goethite. This observation was consistent with previous TEM work showing 2-line ferrihydrite coating of *Gallionella ferruginea* stalks (Ferris and others, 1999). The mixture's metal binding capacity should not be regarded as a direct combination of the independent surface reactivity of the bacteria and iron oxyhydroxide fractions, as masking of bacterial surface reactivity by 2-line ferrihydrite precipitates attributes a non-additive character to the BIOS mixture. Bacteriogenic iron oxides, along with humic and fulvic acids, organic ligands, intact bacteria and mineral colloids, are considered to play an essential role in the control of metal partitioning in pristine and contaminated aquatic environments. Although bacterial surfaces may serve as nucleation sites for mineral oxide colloid formation, their ability to influence metal partitioning as part of the BIOS is attenuated by surface chemical interactions with the iron oxyhydroxide phase.

BIOS CADMIUM SORPTION PROPERTIES

In order to study the metal sorption capabilities of the BIOS, Martinez, Pedersen and Ferris (2004) investigated the interaction of the toxic metal Cd²⁺ with mixture's reactive surface. Their study showed that Cd²⁺ had a greater affinity for the bacteria (organic) fraction of the BIOS. The bound cadmium concentration, at maximum pH values of 8.24 ± 0.31 and 8.39 ± 0.15 , was 0.22 ± 0.01 and 0.56 ± 0.05 μmoles/mg of BIOS for the composite and the bacteria respectively. These values indicate an increase in Cd²⁺ uptake by the organic (bacteria) fraction of more than 2.5 times that of the BIOS composite at similar maximum pH values.

The affinity of Cd²⁺ for the BIOS and its organic fraction was quantified using, discrete pK_{S,j} spectra generated by LPM optimization. As stated earlier in this review, LPM finds an optimum solution set of [B_j] values and assigns them to corresponding pK_{S,j}s, with K_{S,j} defined as *per* equation (2), to generate a discrete spectrum and satisfy the condition $e = |[\text{CdB}^+]_{\text{T,calc,i}} - [\text{CdB}^+]_{\text{T,i}}| = 0$. The best value of [B_j], in μmoles/mg BIOS, is represented by the height of a bar as indicated previously by Martinez, Pedersen and Ferris (2004). Ideally, one [B_j] value should be assigned to a

TABLE 3
Results from pK_s spectrum analysis for Cd^{2+} binding on the BIOS mixture
and the bacterial fraction

Sample	$pK_{s,j(\text{avg})}^a$ ($-\log_{10} K_{s,j}$)	$[B_j]_{(\text{avg})}^b$ ($\mu\text{moles/mg BIOS}$) ^d	$[B]_T^b$ ($\mu\text{moles/mg BIOS}$) ^d
BIOS mixture ($m = 2$) ^c	1.06 ± 0.19	0.04 ± 0.01	0.08 ± 0.02
Bacteria fraction ($m = 3$) ^c	2.24 ± 0.28 0.05 ± 0.12	0.05 ± 0.02 0.29 ± 0.05	0.48 ± 0.06
	1.18 ± 0.02 3.81 ± 0.16	0.11 ± 0.01 0.09 ± 0.02	

^a $pK_{s,j(\text{avg})}$ is the average $-\log_{10}$ of the equilibrium constant for the reaction in equation 8, calculated, from the four replicate values, as defined in equation 9.

^b $[B_j]_{(\text{avg})}$ refers to the individual average site density for each site j and $[B]_T$ is the total site density on the BIOS or bacteria surface, calculated as defined in equation 11.

^c m refers to the number of binding sites on the sample surface resulting from LPM optimization of equation 13.

^dBacteria fraction concentrations were normalized to $\mu\text{moles/mg BIOS}$ using a mass fraction of 0.345 as explained in text.

corresponding $pK_{s,j}$ on the grid. However, in the analysis of the results from replicate data sets, double peaks were observed for a particular site j (Martinez, Pedersen and Ferris, 2004). This occurs because the true $pK_{s,j}$ of the sample fell at an intermediate position between two adjacent $pK_{s,j}$ intervals on the fixed grid (Brassard and others, 1990; Martinez and Ferris, 2001). In order to account for this problem, each doublet was converted to a single peak by averaging the two $pK_{s,j}$ values and computing the weighted average of $[B_j]$. These new quantities along with already existing single peaks, in each replicate spectrum, were then used to calculate overall average $pK_{s,j}$ and $[B_j]$ values ($pK_{s,j(\text{avg})}$ and $[B_j]_{(\text{avg})}$) for each binding site j , as shown in table 3. The total binding site density of the BIOS or the bacteria fraction, $[B]_T$, was computed as $[B]_T = \sum_{j=1}^m [B_j]_{(\text{avg})}$, as expressed by equation (4) above.

LPM was used, as described by Martinez, Pedersen and Ferris (2004) for the analysis of the BIOS Cd^{2+} sorption data. For the mixture, two Cd^{2+} binding sites were found by LPM, with $pK_{s,j(\text{avg})}$ values of 1.06 ± 0.19 and 2.24 ± 0.28 . For the bacterial fraction, three Cd^{2+} binding sites were obtained, with $pK_{s,j(\text{avg})}$ values of 0.05 ± 0.12 , 1.18 ± 0.02 and 3.81 ± 0.16 . $pK_{s,j}$ ($-\log_{10} K_{s,j}$) represents a measure of metal binding strength by reactive surface groups, B_j , where $K_{s,j}$ is defined as *per* equation (2). A decrease in the magnitude of $pK_{s,j}$ would indicate a tighter metal cation binding by functional groups on a reactive solid surface (Martinez and Ferris, 2001).

As mentioned previously, average $pK_{s,j}$ values ($pK_{s,j(\text{avg})}$) were obtained for the BIOS mixture of 1.06 ± 0.19 and 2.24 ± 0.28 , with corresponding binding site densities, $[B_j]$, of 0.04 ± 0.01 and 0.05 ± 0.02 $\mu\text{moles/mg BIOS}$, as summarized in table 3. These values are in good agreement with those of Cd^{2+} sorption studies on pure iron oxide phases. These previous studies have assessed metal oxide interactions using spectroscopic methods and Langmuir isotherm modeling approaches (Cowan and others, 1991; Wang and others, 1997; Randall and others, 1999). Randall and others (1999) reported pK_s values of 2.22 and 2.90 for the Cd^{2+}/H^+ competition reaction: $FeOH^0 + Cd^{2+} \leftrightarrow FeOCd^+ + H^+$. These values suggest weak binding of Cd^{2+} by iron oxyhydroxides (Randall and others, 1999). Cowan and others (1991) mentions constants of 3.10 ± 0.04 and 2.80 ± 0.04 for Cd^{2+} sorption onto $Fe_2O_3 \cdot H_2O$ (am), in the presence of alkaline-earth metal cations. Wang and others (1997), monitored Cd^{2+} binding to sediments composed of amorphous iron oxide and

cryptocrystalline manganese oxide (Wang and others, 1997). Their study reported $pK_{s,j}$ values in the range of 3.73 to 5.47. The results from these studies are comparable to those obtained for the BIOS mixture by Martinez, Pedersen and Ferris (2004) and attest to the low affinity of amorphous iron oxides for Cd^{2+} . Thus, this would suggest that the surface of the BIOS is predominantly composed of mineral oxide reactive sites, and should further imply a lessened efficiency of bacteriogenic iron oxide mixtures for Cd^{2+} retention in contaminated aquatic environments.

The BIOS mixture was subsequently treated with a potent reducing agent to recover the organic component of the composite, as described earlier. Martinez and others (2004), reported average $pK_{s,j}$ values, for the bacteria fraction, of -0.05 ± 0.12 , 1.18 ± 0.02 and 3.81 ± 0.16 with corresponding site densities ($[B_j]$) of 0.29 ± 0.05 , 0.11 ± 0.01 and 0.09 ± 0.02 $\mu\text{moles/mg BIOS}$. These were comparable to $pK_{s,j}$ of -0.80 ± 0.20 , 0.63 ± 0.09 and 2.35 ± 0.10 , and -0.60 ± 0.10 , 0.25 ± 0.19 and 1.93 ± 0.17 for Cd^{2+} binding on intact *Bacillus subtilis* and *Escherichia coli* cells respectively (Martinez and Ferris, 2001). This agreement emphasized the biogenic nature of the BIOS organic fraction and inferred the source of the stronger Cd^{2+} binding by the BIOS bacteria fraction. Comparison of BIOS mixture and bacterial $pK_{s,j}$ values, suggested a higher concentration ($[B_j] = 0.29 \pm 0.05$ $\mu\text{moles/mg BIOS}$) for the high affinity Cd^{2+} complexing reactive site ($pK_{s,j} = 0.05 \pm 0.12$) on the bacterial surface. This observation explained the tighter and greater extent of Cd^{2+} binding by the organic (bacteria) phase of the BIOS mixture.

The two $pK_{s,j}$ values, for the bacteria fraction, of 1.18 ± 0.02 and 3.81 ± 0.16 with corresponding site densities ($[B_j]$) of 0.11 ± 0.01 and 0.09 ± 0.02 $\mu\text{moles/mg BIOS}$ indicate the presence of low affinity Cd^{2+} binding sites on the cell surface. Although the exact chemical identity of these sites would not be accurately determined from LPM analysis alone, the results obtained are consistent with previous models for intact *Escherichia coli* (Gram-) and *Bacillus subtilis* (Gram+) cells where three Cd^{2+} binding sites were found (Martinez and Ferris, 2001). The usefulness of the LPM approach, for the study of toxic metal speciation, can be emphasized by the results obtained by Boyanov and others (2003). In their study, a system of three potential complexing functional groups on the *Bacillus subtilis* surface (COO^- , PO_4^{2-} and SO_4^{2-}) was derived from interpretation of EXAFS measurements in the presence of Cd^{2+} . This finding is consistent with the three binding sites found by LPM on the BIOS organic fraction and previously on intact bacterial cell surfaces. The results of spectroscopic measurements emphasize further the validity of the LPM as a reliable preliminary tool for the study of toxic metal complexation of natural reactive solids (Martinez and Ferris, 2001; Martinez and others, 2004).

Total binding site densities, $[B]_T$, of 0.08 ± 0.02 and 0.48 ± 0.06 $\mu\text{moles/mg BIOS}$ were observed for the BIOS mixture and the bacteria fraction respectively (table 3). This indicated an 83 percent reduction of $[B]_T$ in the BIOS mixture with respect to the bacteria fraction. This pronounced decrease would attest to an increase in reactive site concentration in the bacterial fraction upon removal of the iron oxyhydroxide fraction. The difference in $[B]_T$ values among the two BIOS phases suggested that bacterial cell surface reactive groups were masked from detection by acid base titration. Discrepancies in $[B]_T$ values further confirmed the different chemical nature of the BIOS mixture and organic fraction reactive surfaces (Small and others, 2001; Martinez and others, 2003). In addition, comparison of the $[B]_T$ value from the organic BIOS fraction suggested no effect of residual Fe, on the ability of Cd^{2+} to complex the BIOS bacterial fraction, as $[B]_T$ values are comparable to those of a previous study on Cd^{2+} sorption by intact bacteria (Martinez and Ferris, 2001).

Further interpretation of Cd^{2+} -BIOS interactions may arise from the definition of additivity, as stated earlier (Vermeer and others, 1999). Vermeer and others, 1999

suggest that the overall adsorption of a metal ion to a mixture such as the BIOS will be smaller than predicted by the additivity rule when the metal ion shows a greater affinity for the organic rather than the iron oxide fraction (Vermeer and others, 1999; Small and others, 2001). In this study, for comparable initial Cd^{2+} concentrations, the total bound metal, $[\text{CdB}^+]_{\text{T},j}$, at maximum pH values of 8.24 ± 0.31 and 8.39 ± 0.15 , were 0.22 ± 0.01 and 0.56 ± 0.05 $\mu\text{moles/mg}$ of BIOS for the mixture and bacteria fractions respectively. These values suggested a higher affinity of the bacteria for Cd^{2+} and, in consequence, a deviation of the BIOS mixture from the additivity rule. This further indicated a degree of bacterial cell surface functional group masking by iron oxyhydroxides, as mentioned previously (Vermeer and others, 1999; Small and others, 2001).

The application of LPM provided useful insight into the interaction of Cd^{2+} with the BIOS mixture and its bacteria fraction. Differences in solid surface reactivity were observed through the interpretation of LPM optimized parameters such as $[\text{B}_j]$, $[\text{B}]_{\text{T}}$ and measures of Cd^{2+} binding strength, $\text{pK}_{\text{s},j}$. The complicated nature of the BIOS surface should require the use of direct spectroscopic methods, for the identification of Cd^{2+} complexing functional groups, such as those employed by Boyanov and others (2003). For this reason, LPM assumed a 1:1 Cd^{2+} - B_j binding ratio to j non-identified surface binding sites. The agreement, as described earlier, between the results of Boyanov and others (2003) and those of LPM for the BIOS organic phase strongly suggest that LPM is a useful preliminary tool for the determination of the number and concentration of binding sites, B_j , on the BIOS surface, along with their Cd^{2+} binding strengths, $\text{pK}_{\text{s},j}$. Comparison of parameters such as, $[\text{B}]_{\text{T}}$, showed a lower reactive site concentration on the BIOS mixture's surface than that on the bacteria fraction. This indicated the non-additive character of the BIOS end-member components and the masking of bacterial surface functional groups by iron oxyhydroxide precipitates. Calculated apparent equilibrium constants, $\text{pK}_{\text{s},j}$, showed that Cd^{2+} had a stronger affinity for the BIOS bacteria fraction. These conclusions portrayed LPM as a useful tool for the analysis of the metal and proton sorption abilities on a wide range of natural reactive solids, such as humics, organic ligands and mineral oxide colloids. These, along with BIOS mixtures, play a major role in the control of metal partitioning in contaminated and pristine environments.

As suggested in a previous study, the BIOS are bound to have uncertainties in size, geometry and penetrability by counter ions (Martinez and others, 2003). Although electrostatic effects are important in metal-colloid interactions, an attempt to calculate these intrinsic parameters using best fit routines such as linear programming or non-linear least squares, could result in increased flexibility of the optimization method. This, in turn, may introduce uncertainty in optimized electrostatic parameters, limiting their contribution to the understanding of their originally intended chemical meaning.

CONCLUSIONS

Proton and cadmium sorption speciation calculations on the BIOS and organic phase surfaces provided detailed information regarding the number of metal complexing sites and their binding strength for the different BIOS phases. These results suggest that both FOCUS and LPM are useful preliminary tools for the assessment of the metal and proton binding properties of naturally occurring sorbent materials. The studies were also able to conclude that the BIOS could serve as a natural means of bioremediation due to its capacity to sorb and retain toxic metal cations in contaminated aqueous environments. The natural abundance of this material in freshwater systems should serve as a monitor of the increments in toxic metal cation concentrations. In order to better understand and improve the efficiency of the BIOS metal sorption, the results of these studies should be complemented through the use of spectroscopic methods to characterize iron oxide structure and atomic metal binding properties on the iron

oxide and organic surfaces. For this reason, the reader is referred to the works of Kennedy and others, (2003a, 2003b, 2003c) and Boyanov and others, 2003 for a sound application of spectroscopic methods to the analysis and structural characterization of iron oxides and toxic metal binding properties, in the oxide phases and in oxide/organic matter mixtures.

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