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Biogeochemical controls on metal behaviour in freshwater environments

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Abstract

The biogeochemical controls on metal behaviour in aqueous environments involve complex linkages of biological, principally bacterially driven, and geochemical processes, which occur at both microscopic and macroscopic scales. The framework of aqueous surface chemistry and aquatic geochemistry continues to provide the foundations of the emerging paradigm: (1) metal behaviour (e.g., transport, toxicity, bioaccumulation) is governed by solid-solution reactions; (2) pH, ionic strength, redox potential, the types and concentrations of solution elements, and solid surfaces all interact to determine metal behaviour in any given system; (3) metal sorption reactions show both metal ion and solid surface specificity; (4) sorption reactions are dynamic and reversible; and (5) processes are at sufficient pseudo-equilibrium or dynamic steady state that thermodynamics can be applied to describe such reactions.

Reactions controlling metal behaviour are increasingly modelled, with some success, using a variety of geochemical modelling approaches all based on this framework. However, not yet considered in the majority of these thermodynamic treatments of metal dynamics is that these reactions are highly influenced by biological factors, which will affect their location, magnitude and rate. The extent of this influence will be largely driven by microbial ecology, and thus, a fundamental identification and mechanistic understanding of how these factors will drive the geochemistry of a particular system is required. The lack of substantive biogeochemical understanding stems from the fact that the field of environmental microbiology, with its crossover to environmental geochemistry, has only recently begun to receive attention. The developing evidence strongly underscores the impact of bacterial reactions for a number of highly relevant processes related to metal dynamics such as solid solution partitioning, mineral precipitation and dissolution reactions, and intense changes in system geochemical conditions.

The development of new molecular level microscopic and spectroscopic techniques provides powerful tools to promote an integrated approach to understanding the mechanisms underlying metal dynamics, which encompasses both the geochemical and biological components of this dynamic and complex cycle. Particularly, when used in conjunction with new molecular biological tools, these multiple lines of evidence will provide a mechanistic model of the controls on metal behaviour that will reflect the inherently complex reality of natural systems. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: metals; freshwater; sorption; bacteria; biogeochemical controls; molecular techniques; XAFS; XANES; geochemical modelling

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1. Introduction

Understanding the dynamics of metal behaviour in freshwater environments has been a major focus of environmental geochemists for several decades, and interest in this area continues to grow as regulatory bodies are faced with the myriad complexities of how to regulate, mitigate and remediate contaminated water bodies. Trace metal contamination of aquatic environments is of critical concern due to potential detrimental effects both directly within foodwebs of impacted systems, as well as obvious linkages to human health concerns associated with contaminated drinking water and fish supplies. Remediation remains a key challenge due to our incomplete understanding of the complex behaviour of metals in aquatic systems. Metals can exist in a variety of forms in water, not all of which are equally toxic, mobile, or bioavailable. Further, metal associations are dynamic and reversible, reflecting changing physico-chemical conditions. Therefore, even with reduced loading scenarios, potential effects from metals released from sedimentary compartments can be envisioned for some time to come.

A necessary precursor to the determination of successful remediation strategies is a fundamental understanding of the key processes and dominant variables controlling metal dynamics, as well as the interplay amongst those variables and processes. Water is by nature an inherently multi-disciplinary topic. Thus, any real understanding of controls on metal behaviour in aquatic environments must identify and quantify the potential interactions and linkages amongst the traditionally sectarian disciplines of chemistry, physics, biology and geology. What has become increasingly evident from the vast but segregated literature on the broad topic of metals in aquatic environments is the trans-disciplinary nature of the controls on metal cycling. While substantial literature exists within each of these disciplines on various aspects of the topic, for the most part potential linkages amongst influencing parameters have not been well identified or examined; although these types of syntheses are beginning to appear in the literature (e.g., Ledin, 2000). The focus for the 21st century, therefore, must be the interplay amongst these key areas. In this review, we discuss the important concepts that have emerged over the last 30

years, the approaches taken in both laboratory and field studies and provide some suggestions for future research in this emerging field.

2. Surface chemistry and reactivity of natural materials

2.1. Surface reactions

A foundation of the emerging aquatic metal biogeochemistry paradigm lies in the discovery that reactions occurring at solid surfaces play a dominant role in determining metal partitioning between the solid and solution phases, and thus, the potential impact of metals in aqueous environments (Jenne, 1968). Interfacial reactions are particularly important in the water column where, generally, system conditions are undersaturated with respect to mineral phases and thus precipitation is not commonly a significant process for solid metal formation. In contrast, precipitation of metal phases can be the dominant process in sedimentary compartments where solubility constraints are more commonly overcome.

The overwhelming importance of interfacial reactions has driven the development of surface geochemical modelling techniques, discussed in more detail subsequently (see Section 5). At the forefront of these techniques has been the “surface complexation” approach. The success of surface reaction-based geochemical modelling is predicated upon two characteristics of interfacial reactions involving binding sites at the surface of the solid and solution ions such as metals: (1) systems are at sufficient pseudo-equilibrium that thermodynamic equilibria can adequately describe such reactions; and (2) sufficient consistency to metal–surface site interactions exists across the gamut of solids and metals that occur in natural waters to permit generalities to emerge.

2.2. Surface reactivity

Reactions occurring at the interface of solid surfaces with their surrounding aqueous medium, stem from the fact that solids carry a surface charge in aquatic environments and thus attract counter-ions from solution to balance that charge (see Fig. 1 for an oxide surface as an illustrative example). The

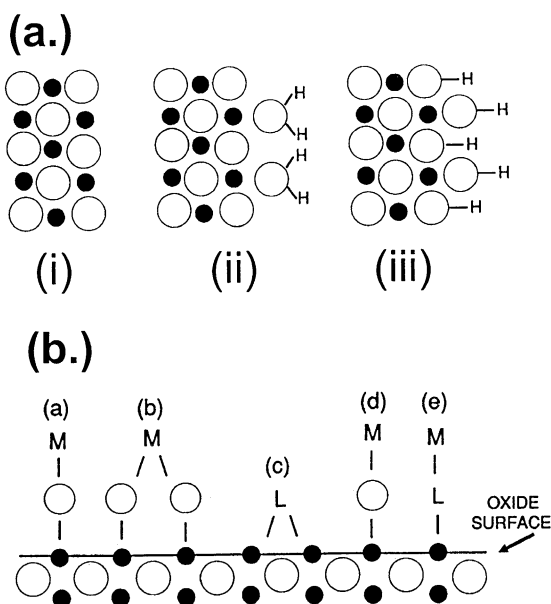


Fig. 1. (a) A cross-section of the surface layer of a metal oxide: ●, metal ions and ○, oxide ions. (i) A dry oxide surface; (ii) in the presence of water, surface metal ions may coordinate H₂O molecules; (iii) dissociative chemisorption leads to a hydroxylated surface. (b) Possible hydrated oxide surface complexation reactions. Oxide surface: ●, metal ions; ○, oxide ions; and solution ions: M, metal ion; and L, ligand. The surface hydroxyl group (OH) has a complex-forming O donor atom that can complex a metal ion from solution (a); two surface O donor atoms can complex one metal ion (b); the underlying metal ion (● e.g., Fe, Mn, Al, Si), can exchange its surface OH group for other ligands (c); the O donor atom can complex a metal–ligand complex (d); or (e) the underlying metal ion can exchange its OH group for a ligand–metal complex (adapted from Warren and Zimmerman, 1993; with permission*).

potential for reactions involving metals to occur at solid surfaces arises from: (1) the existence of unfilled charge requirements of molecules or functional groups (e.g., hydroxyl, carboxyl, phosphoryl) at the solid surface; (2) the unique properties of water itself that cause it to be a polar molecule; (3) the presence of solution or dissolved elements that also possess non-neutral charges; and (4) the inherently dynamic nature (strongly driven by biological processes) of aquatic systems that causes constant disequilibrium within the system and the impetus for reactions to occur.

Dependent on their respective dissociation constants (i.e., pK_a), functional groups at a solid surface

can become negatively charged through the loss of protons (in response to changes in system chemistry that affect pH, such as surface functional groups buffering pH by donating protons to solution as solution pH values increase). The deprotonated functional group can then subsequently act as a binding site for positively charged ions such as metals. Mineral surface functional groups can also become positively charged through association of additional protons, thereby buffering pH by accepting additional protons at lower pH values. Protonated functional groups are capable of binding negatively charged compounds under low system pH conditions. Thus, mineral solid surfaces are often amphoteric and can potentially bind both cations and anions dependent on their surface charge, which ultimately depends on system pH.

The exact pH at which a solid surface becomes negatively charged depends on the associated pK_a values of its surface functional groups. Each type of binding site, e.g., carboxylic or phosphoryl on organic solid surfaces or hydroxyl on mineral surfaces has a specific dissociation constant, and thus net particle surface charge, whether positive or negative, is a function of the particular solid involved. In general, any reaction involving a solution ion with a solid surface functional group that results in a surface association being formed is referred to as a sorption reaction. Sorption includes both covalent bonds and electrostatic complexes, which differ in the strength of their associations with the solid surface. Sorption reactions in which a covalent bond is formed between the solute ion and the surface species are termed inner sphere complexes. The term “adsorbed” is often used to denote the formation of an inner sphere complex. Adsorption is the result of both chemical and electrostatic interactions. In contrast, outer-sphere complexes are those in which the solute ion and surface species are attracted by electrostatic forces alone. Solutes sorbed as outer-sphere complexes are more weakly associated to the solid surface, and thus, are more easily re-solubilized.

2.3. Controls on surface reactions

The underlying control on solid surface charge as well as on the speciation of elements within solution

is pH. As such, pH dictates cation and anion partitioning between the solid and the solution phases with all the attendant ramifications for geochemical behaviour, e.g., mobility, reactivity, bioavailability, bioaccumulation and toxicity. pH is commonly thought of as the master variable controlling metal behaviour in aquatic environments.

While pH has an overwhelming dominance in metal dynamics, other factors can also play a substantial role within this framework. The factors that can profoundly affect the snapshot of metal behaviour observed at any given time are, specifically, (1) the physical and chemical properties of an aquatic environment (e.g., ionic strength, redox status, presence of water currents, the location and activity of biota); (2) the composition of its solution phase (e.g., the types and concentrations of solution species); (3) the types and densities of solid sorbents and the kinetics of relevant reactions, e.g., (the influence of biological activity and temperature) (Allard et al., 1987; Sigg, 1987; Westall, 1987; Sheintuch and Rebhun, 1988; Tessier et al., 1989; Johnson, 1990; Warren and Zimmerman, 1994a,b; Warren and Ferris, 1998; Warren et al., 1998; Small et al., 1999, 2000).

Ionic strength affects metal partitioning through two principal mechanisms. One, it effects the activities of species in solution: higher ionic strength leads to decreased activities or effective concentrations of elements to participate in reactions. Two solution ionic strength also affects the thickness of the interfacial, charge differential zone between the solid surface and the bulk solution: higher ionic strength decreases the depth of that zone. Thus, in general, higher ionic strength leads to a decrease in the number of surface complexation reactions occurring between metal species in solution and functional groups at solid surfaces (e.g., Puls et al., 1991; Lores and Pennock, 1998). In particular, outer-sphere complexes, those based on electrostatic interactions, are more inhibited at high ionic strength than are inner-sphere complexes.

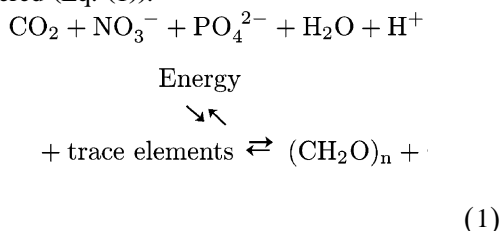
Redox status influences metal dynamics in a number of ways. Redox species of metals that possess more than one possible oxidation state in natural waters are differentially mobile, soluble, toxic and reactive (Ahmann et al., 1997; Losi and Frankenberger, 1997; Newman et al., 1998; Brown et al.,

1999a,b; Wang, 2000). Highly charged metals ions, such as Cr(VI), As(V) and Mn(VI) tend to exist as oxyanions in solutions at moderate to high pH, and tend to form covalent bonds. Due to their high charge, such ions experience a greater attraction to negatively charged surface sites and therefore, these ions can behave as potential toxins in aqueous systems due to their affinity for biological binding sites. For example, hexavalent chromium, the common pollutant moiety, is more toxic than trivalent chromium, the naturally occurring form (Bartlett, 1991; Richard and Bourg, 1991; Baruthio, 1992). Bacteria are able to enzymatically reduce toxic metals; the reduction is used as a detoxification process (e.g., Hg^{2+} to Hg^0 ; Saouter et al., 1994). Bacteria also enzymatically reduce metals within mineral phases, such as Fe and Mn oxyhydroxides. A significant proportion of the metal reduction occurring within sediments is thought to be a result of such enzymatic processes (Lovley, 1995). Biological reduction can also lead to changes in state. For instance, the microbially mediated reduction of soluble Hg(II) to volatile Hg(0) has been identified as a potential strategy to stimulate volatilization and removal of mercury from contaminated environments (e.g., Ogunseitan and Olson, 1991). All of these redox-mediated transformations have differential implications for metal behaviour in aquatic systems.

System redox status can also affect the partitioning of elements between the solution and solid phases. For instance, iron in its oxidized trivalent state, Fe^{3+} readily hydrolyzes and precipitates as an oxyhydroxide solid Fe(III) phase that then subsequently provides a highly reactive and important surface for trace metal scavenging in aqueous environments. Reduction of Fe^{3+} to divalent Fe^{2+} can be accompanied by dissolution of the solid Fe oxyhydroxide phase and potential release of any associated metal. Similarly, oxidation and reduction involving Mn will result in solid phase capture or release to solution. However, in contrast to Fe oxidation that can proceed abiotically, oxidation of Mn(II) to Mn(IV) is principally a microbially mediated process (Emerson, 2000).

Further, it must also be kept in mind that the redox status of the compartments (e.g., water column, sediments) of any given system is directly impacted by the biological composition and function

within those compartments. Biota influence system redox status principally through the two major, linked processes of photosynthesis and decomposition that cycle carbon and energy through organic and inorganic states. Biota, therefore, affect both oxygen and proton concentrations. The links between redox and pH become evident when these two processes are considered (Eq. (1)).



Primary production in the presence of either electromagnetic (photosynthesis) or chemically derived energy drives the reaction to the right producing organic, reduced carbon compounds; consuming protons; and producing molecular oxygen as a byproduct. Decomposition will drive the reaction to the left, oxidizing organic carbon compounds through a series of organic intermediates, potentially back to CO_2 ; consuming oxygen in the process; and liberating protons as a by-product. The exact stoichiometry of the reaction depends on system conditions, the nature of the inorganic (e.g., HCO_3^- , CO_3^{2-} , CO_2) and organic carbon sources (e.g., lactate, pyruvate, succinate), and the autotrophs involved. It is important to keep in mind that the relative intensities of these reactions, especially photosynthesis, are both diurnally and seasonally correlated. Therefore, biological reactions influence system chemistry in a dynamic manner, whether viewed on a daily or on a seasonal basis. The relative impact of these reactions will be determined by both the intensity of the reaction rates, which is a function of biotic density and activity, and by the location of biota in the system. Thus, at periods of high biological activity, significant changes in redox or pH may occur in localized areas that can strongly impact metal dynamics within that zone.

3. Metal speciation and reactivity

Not all metals are equally reactive, toxic, or available to biota (Hare, 1992). In fact, measurement of

total metal ion concentration in solution is often meaningless for prediction of metal behaviour (Campbell and Tessier, 1989). Rather, the free ion form is thought to be the most available and toxic (Sunda and Lewis, 1978; Luoma, 1983; Martell et al., 1988; Shuttleworth and Unz, 1991; Campbell, 1995). With regards to reactivity, it is generally observed that different metal ions display differing affinities for surface binding sites across substrates, and further that not all metals display the same affinities for individual surfaces (e.g., Sauvé et al., 2000). The strength of the metal-sorbent bond is given by a surface complexation/binding constant, $K_{\text{Mz}+}^{\text{S}}$ (where M^{z+} refers to the specific metal of interest). The value of the binding constant has important implications for potential re-release of the metal ion into solution given changes in system chemistry. The greater its surface complex formation constant, the less likely that the metal ion will be desorbed into solution, where it is potentially more available, reactive and toxic. That differences in the behaviour of various metals under similar conditions are likely and often observed, dictates that the specific metals of interest must be specified for accurate prediction, and precludes the applicability of a general model of metal–solid interactions.

The speciation or dissolved forms of a metal in solution is of utmost importance as it influences the partitioning of the metal between the solid and solution phases, the extent of biological incorporation of the metal, and its toxicity. In most cases, the bioavailability and toxicity of the free hydrated ion is greater than that of metals complexed with other ligands (Luoma, 1983; Dzombak et al., 1986; Lovgren and Sjöberg, 1989; Martell et al., 1988). For example, dissolved ligands such as organic compounds can effectively compete with solid surfaces for solution metal ions, forming dissolved metal complexes, which keep metals in solution. Metal speciation is governed by the same parameters governing surface complexation reactions, i.e., pH, redox, types and concentrations of solution compounds, stability of different metal-ligand forms, concentrations of competing cations, temperature and reaction kinetics. The hydrolysis constant for a given metal is the most important parameter to establish for prediction of a metal's speciation at a given pH, and thus, its likely behaviour. This is because the

values of the hydrolysis constant and the surface complex formation constant for a given metal with a specific sorbent have been shown to be highly correlated; the greater the value of the hydrolysis constant, the greater the affinity of the metal for solid surfaces. This correlation holds for various metals with Fe oxyhydroxides (Dzombak and Morel, 1990), silica and bacterial surfaces (Warren and Ferris, 1998).

Metals can be classified in a number of ways. One classification is as essential or as non-essential to living organisms. Those metals generally considered to be essential are Na, K, Mg, Ca, V, Mn, Fe, Co, Ni, Cu, Zn, Mo and W. Those metals that are considered to be non-essential are usually toxic to organisms at very low levels and include Ag, Cd, Sn, Au, Hg, Tl, Pb, Bi, Al, and also the metalloids, As, Sb and Se (Beveridge et al., 1997). Metals are also classified according to the oxidation state of their ions and/or their position in the periodic table. Monovalent alkali metal cations and divalent alkaline earth metal cations are characterized by low reactivity and high relative solution concentrations in freshwater systems. In the solution, these cations are bound predominantly with major inorganic ligands. Monovalent cations are generally sorbed to a greater extent with increased pH. This indicates that these ions act as counter-ions for surface charge developed with pH changes; the association of the ion with the sorbent is likely as an outer-sphere complex (Stumm, 1992; Stumm and Morgan, 1996). Divalent alkaline earth metals are also considered to act as counter-ions. As a counter-ion, Ca^{2+} has been shown to be more strongly complexed by the surface than Na^+ (Schiewer and Volesky, 1997a). The complexation of these ions affects the stability of the interactions between other metal ions and the sorbent surface sites, the macromolecular conformation of the surface and colloid coagulation (Buffle, 1990).

D-block (groups 3–12 of the periodic table) divalent cations are not considered to enter into the same type of surface complex as the alkaline earth metals but are more strongly bound, inferring the formation of an inner-sphere complex (Dzombak and Morel, 1990). Sorption of such divalent cations is characterized by a rapid increase in adsorption to surfaces over a small pH range. Divalent cations are also sorbed preferentially over protons at binding sites on

organic ligands where chelating effects can occur (Stone, 1997). D-block divalent cations have a strong affinity for both “hard” (tending to form electrostatic bonds) and “soft” (tending to form covalent bonds) ligands (Buffle, 1990). Highly charged, trivalent and tetravalent, D-block cations, are more complicated still, in that they often tend to have the ability to exist in more than one oxidation state. Some of these ions, such as Al(III) and Fe(III) tend to precipitate as metal hydroxides. They have affinities for both soft and hard ligands, but have a higher affinity for soft ligands such as fulvic acids (Buffle, 1990). Binding of the highly charged metals with such ligands effectively removes them from solution to the sediment. Highly charged ions will, however, interact with the hard ligands for which the divalent cations have a higher affinity.

4. Important solids

4.1. Fe and Mn oxyhydroxide minerals

To a large extent, mineral surfaces, especially those of Fe oxyhydroxides, have received a large degree of attention in aquatic metal chemistry. This is due to their ubiquitous and abundant nature, as well as their proven geochemical reactivity (Honeyman and Santschi, 1988; Brown et al., 1999a,b). Metals can be incorporated into solid minerals by a number of processes including: surface reactions such as ion exchange reactions (non-specific adsorption) and specific adsorption to surface hydroxyl groups (surface complexation), coprecipitation (solid solution), and precipitation as the discrete oxide or hydroxide (Martinez and McBride, 1998). Further, it is common that Fe and Mn oxyhydroxides form surface coatings on other types of mineral substrates such as clays and carbonates (e.g., Whitney, 1975; Helios-Rybacka and Forstner, 1986; Warren and Zimmerman, 1993, 1994c; Coston et al., 1995) and thus, while their relative mass in a suspended sediment pool may be relatively small, they often dominate the geochemical reactivity of that sediment compartment.

There are a number of factors that can influence the extent of sorption by any one mineral sorbent

type, such as: degree of crystallinity (e.g., Martinez and McBride, 1998), number of defect sites, impurities and/or coprecipitates (Banfield and Hamers, 1997; Webster et al., 1998), surface coatings of organics and/or bacteria, and particle size (Brown et al., 1999a,b). While Fe oxyhydroxides surfaces continue to be characterized, modelled, and understood, attention is increasingly turning to other minerals, which are also likely to play a role in determining the geochemical behaviour of metals in aquatic environments through their similar characteristics and potentially significant concentrations in certain locales. It is important to evaluate the relative reactivities of different solids, because while Fe oxyhydroxides may be more abundant than other minerals such as Mn oxyhydroxides in some environments, their surface reactivities may be lower (e.g., Tessier et al., 1996; Nelson et al., 1999a). In some cases, minerals present in lower mass quantities than Fe oxyhydroxides may actually provide greater sorptive capacities through higher surface reactivities relating to higher energy binding sites and/or higher surface area to volume ratios, and thus higher densities of surface binding sites (Nelson et al., 1995, 1999a). The overall ability of any sorbent to scavenge or sorb metals reflects not only the concentration or mass of that substrate available, but also its surface reactivity or ability to sorb metals.

4.1.1. *Fe oxides*

As mentioned above, most of the quantitative research effort in aqueous surface chemistry to date has focused on one important, geochemically reactive mineral solid, Fe(III) oxides. The interest in Fe oxides arose due to their ubiquitous and abundant nature. Iron's prevalence and occurrence in the hydrosphere, lithosphere, biosphere and pedosphere are explained by it being the seventh most abundant element in the earth's crust. There are 16 Fe oxides (Cornell and Schwertmann, 1996), which include oxides, hydroxides, and oxide hydroxide forms. These are often collectively referred to as the iron oxyhydroxides, are very stable (i.e., exhibit low solubility), and possess high energies of crystallization. Fe oxides are thus frequently formed as very small precipitates with high associated surface area to volume ratios. It is their high associated surface areas, which make these minerals highly reactive.

All Fe oxides contain Fe(III) and O or OH groups; however, these oxides vary in their mineralogical composition and structure, as well as their occurrence and relative densities in various compartments of the geosphere. Further, individual Fe oxides frequently have several polymorphs, which will all display varying reactivity due to differences in surface characteristics. Most Fe oxides exhibit crystalline form with one notable exception, ferrihydrite, which is of particular interest due to its common existence in aquatic environments. Ferrihydrite ($\text{Fe}_5\text{HO}_8 \cdot 4\text{H}_2\text{O}$) is typically poorly ordered and will, unless stabilized, ultimately transform into a more stable member of the Fe oxide group; the end member of which is commonly goethite. Frequent reference to amorphous iron oxides or oxyhydroxides and hydrous ferric oxide (HFO) occurs in the literature and these terms should all be considered synonymous with ferrihydrite (Cornell and Schwertmann, 1996). Other Fe oxides found to occur in aquatic environments include lepidocrocite ($\alpha\text{-FeOOH}$), schwertmannite ($\text{Fe}_{16}\text{O}_{16}(\text{OH})_y(\text{SO}_4)_z \cdot n\text{H}_2\text{O}$), magnetite (Fe_3O_4), hematite ($\alpha\text{-Fe}_2\text{O}_3$) and goethite ($\alpha\text{-FeOOH}$).

Fe oxyhydroxides are likely to occur in almost all aquatic environments in significant quantities. Thus, they are also likely to play a substantial role in influencing metal behaviour, a role that has been well documented for several environments (e.g., Honeyman and Santschi, 1988; Brown et al., 1999a,b). Application of the surface complexation model (SCM) to predict the surface reactivity of Fe oxides for metals has been widespread. Dzombak and Morel (1990) used the SCM when compiling the most complete quantitative study to date of the surface reactivity of HFO (the SCM approach is discussed in more detail subsequently in Section 5). However, the variety of Fe oxyhydroxides that occur in natural environments and their weathering and dissolution potentials precludes accurate prediction of their likely reactivity in any given environment using one general molecular formula (Perret et al., 2000). The exact oxide involved, its degree of crystallinity, associated impurities, size, presence of coprecipitates, extent of weathering, and associated surface materials will all act in concert to affect the relative reactivity of that oxide surface and therefore its ability to sorb metals.

4.1.2. *Mn oxides*

The sorptive capacity of Fe oxyhydroxides is extremely well studied relative to other potentially relevant mineral surfaces. Currently, a developing interest in reactions occurring at other potentially important naturally occurring mineral surfaces such as Mn oxyhydroxides is evident (e.g., Tebo et al., 1997; Nelson et al., 1996, 1999a). Mn(III,IV) oxides are generally denoted as MnO_x ($1 \leq x \leq 2$) and include oxides, oxyhydroxides and manganates (contain a variety of charge balancing, low valence cations; Tebo et al., 1997). Like Fe oxides, Mn oxides are also very stable, exhibit low solubility (Stumm and Morgan, 1996), and precipitate as very small crystals that are often poorly crystallized or amorphous (Fortin et al., 1993; Mandernack et al., 1995; Tessier et al., 1996), and thus also possess very high surface reactivities. Mn is also one of the more abundant elements observed in freshwater environments, as it is the second most abundant transition metal in the earth's crust. Mn oxyhydroxides have been shown to be highly effective scavengers of a variety of metals (e.g., Tessier et al., 1996; Fuller and Harvey, 2000) and are thought to be potentially more reactive than Fe oxides (e.g., Whitney, 1975; Balikungeri and Haerdi, 1988; Bendell-Young and Harvey, 1992; Nelson et al., 1999a). However, in contrast to Fe oxyhydroxides, the oxidation of Mn is thought to be a biologically controlled process under circumneutral conditions in most freshwaters (Nealson et al., 1989; Sunda and Kieber, 1994).

4.2. *Natural organic matter (NOM)*

Natural aquatic systems contain a variety of solids other than minerals such as Fe or Mn oxyhydroxides that may be geochemically important substrates for metal transport, capture and fate. In particular, organic solids such as natural organic matter (NOM) or such NOM components as humic and fulvic acids (HA, FA), and bacteria have all been shown to be effective scavengers of trace elements and thus important components to consider in any globally applicable model of trace element behaviour. NOM molecules are polyelectrolytes, or macromolecules

consisting of individual moieties that each possess a high density of electrical charges (Buffle, 1990). In contrast to oxide mineral surfaces that possess only one type of functional surface group, organic compounds are highly complex, highly variable compounds that may possess varying quantities of a number of different types of surface functional groups. Further, while mineral surface hydroxyl functional groups show an effective sorptive capacity that spans a fairly narrow pH range (usually between 6 and 8; Dzombak and Morel, 1990), organic surfaces can be effective sorbents at much lower pH. This sorptive capacity is due to the associated pK_a s of the functional groups comprising the organic solid, particularly carboxylic groups ($pK_a \sim 4\text{--}6$).

4.2.1. *Definition and operational characterization of NOM*

NOM comprises all detrital organic matter in a reservoir or natural ecosystem, specifically excluding living organisms and compounds of man-made origin (Buffle, 1990). NOM is a significant component of the majority of freshwater environments. There are a variety of potential NOM sources including primary autochthonous productivity (e.g., internal production), allochthonous or external inputs to the system, contributions from littoral shore zones, and release of sediment organic matter.

Aquatic NOM has traditionally been categorized into a number of operationally defined fractions based on several, sometimes overlapping, criteria. Dissolved and particulate organic matter (DOM and POM) are defined operationally on the basis of size as the organic material that either passes through (DOM) or is retained (POM) by 0.45- μm pore filters. Humic substances (thought to comprise both humic, HA, and fulvic acids, FA) are operationally defined as the refractory fraction of NOM, on the basis of lability (e.g., Frimmel, 1998), and can occur in both the dissolved and particulate size classes. Humic substances are characterized by yellow colour, intensive UV-absorbance, polydispersity, high molar mass ($10^3\text{--}10^5$ g/mol) and polyfunctionality (Aiken et al., 1985), and are generally present at concentrations comparable to inorganic salts, commonly reaching values as high as 5 mg C/l in aquatic systems (Egeberg et al., 1999).

Fulvic and humic acids (FA and HA) originate ultimately from the pedogenic or soil compartment of a particular aquatic environment's catchment. Thus, the types and concentrations of FA and HA in any aquatic environment will be highly dependent on the nature of the soils draining the watershed. Humus is thought to be formed as a result of two processes, degradation of plant tissue residues, and condensation reactions of smaller molecules (Buffle, 1990; Cabaniss et al., 2000). Degradation of plant tissue is a microbially controlled process that leads to subsequently more oxidized and less polymerized end products. For example, plant tissue can be degraded through a series of increasingly labile substances from humic compounds to fulvic compounds, and ultimately to CO₂. Humic substances derived from decaying plant tissues, primarily lignins and cellulose (Morel and Hering, 1993), retain a high degree of aromaticity from their precursors, which has implications for metal reactivity. The alternative, non-biologically mediated process is the oxidation and condensation or aggregation of small organic molecules to form more complex polymerized macromolecules of humic and fulvic acids (Buffle, 1990). Such heteropolycondensates have as their building blocks: polysaccharides, peptidic material, *N*-acetylamino sugars, and polyphenolic material (Christy et al., 1999). HA and FA are distinguished from each other operationally by their solubility. HA is defined as the fraction of pedogenic organic matter, which is soluble in both acids and bases, while FA is soluble only in bases (Buffle et al., 1984). As indicated above, FAs are derived from degradation of HAs and thus are usually smaller in size, more labile and contain lower numbers of aromatic functional groups. Terrigenous humic compounds are usually close to 50% carbon by weight (Morel and Hering, 1993). Buffle (1990) suggests that HA is 56.2% carbon by elemental composition and FA approximately 45.7% carbon. Fulvic compounds generally represent the largest NOM fraction in freshwaters (40–80%), while humic acids represent only 5–10% of NOM (Buffle et al., 1998). However, these values are highly uncertain. Non-humic organic carbon has been reported to make up anywhere from 20–60% of the total organic carbon pool in aquatic environments (Boerschke et al., 1996; Hamilton-Taylor et al., 1996). Ultimately, the types and con-

centrations of NOM are highly variable across systems. Potential differences in the organic pool will likely translate into differing relative reactivities and affinities for metal contaminants.

4.2.2. *NOM reactivity*

Any attempt to describe the reactive characteristics of aquatic NOM must account for the three major complexities of such molecules: the polyelectrolyte nature of the macromolecules; their intrinsic heterogeneity, reflecting, as indicated above, their nature as intermediate steps in processes of molecular transformation; and thus, the lack of a unique molecular structure and therefore associated weight (Cabaniss et al., 2000). There is, as outlined above, a general relationship between physical size of organic compounds and their lability. Small organic molecules are usually more labile and possess both higher turnover and assimilation rates, and lower residence times. However, within any operationally defined size class of NOM, there may be both labile organic constituents as well as HA. Thus, all measured properties of the NOM fractions, such as molecular weight, are in fact governed by statistical distributions (Tanford, 1961). This complexity is handled by considering a distribution of model structures representing the variety of compounds that exist within any defined organic pool, and expressed as the number- and weight-averaged molecular weights of a NOM sample (Chin et al., 1994). It is the ratio of weight-averaged molecular weight to the number-averaged molecular weight that gives the polydispersity or relative partitioning of NOM (Cabaniss et al., 2000).

4.2.3. *Acid–base behaviour of NOM*

NOM is a heterogeneous, complex mixture of many organic compounds, which taken together have acidic character and considerable buffering capacity (Takács et al., 1999). The two dominant categories of HA functional groups are carboxyl molecules, which have been estimated with calorimetric techniques to represent 60–90% of the acid groups (Perdue, 1978) and phenolic groups. The reactivity of humic material is often best characterized by a distribution of affinity constants. This is a direct consequence of the complex heterogeneous nature of the

material and its associated spectrum of functional groups. Not all functional groups are chemically equivalent; thus, each type of surface functional group or binding site, can in principle, have its own unique intrinsic chemical affinity for protons and metals. Perdue (1985) summarized the pK_a values for model organic ligands that might be incorporated in NOM. The acidic groups vary from sample to sample, but they consist mainly of carboxyl with pK_a values from ~ 3 to 6, and phenolic with pK_a s from about 9 to 11. The intermediate pK_a values between 5 and 9 could be attributed to a wide variety of functional groups, including β -dicarbonyl compounds, enols, and alcohols, as well as surface sites on inorganic substrates (Smith and Kramer, 1999b).

4.2.4. Acid–base behaviour: comparison of organic and mineral surfaces

An important distinction between organic (NOM and bacteria) and mineral surfaces is their overall surface charge in the pH range of aquatic environments. The surface hydroxyl groups of Fe and Mn oxide tend to deprotonate around pH 7. Oxide surfaces can therefore carry an overall net positive, negative or neutral charge. Thus, Fe and Mn oxides will typically sorb cationic species at higher pH and anionic species at lower pH values. In contrast, NOM and bacterial surfaces possess a number of different types of surface functional groups, which can deprotonate at a variety of pH values from low (carboxylic, ~ 3 –5) to high (amine, ~ 9 ; phenolic, ~ 10). Therefore, in the pH ranges of natural systems, NOM and bacterial surfaces are always negatively charged and show a high affinity for positively charged solid surface functional groups or cationic metal species.

These differences in surface characteristics are clearly manifested in acid–base titrations of oxide and various organic surfaces (Fig. 2). Surface associated charge versus pH for a mineral Fe oxide surface indicates that Fe oxide surfaces will become net negatively charged at pH values above the pH_{zpc} (i.e., the pH at which the oxide surface has zero net surface charge) and net positively charged at pH values below the pH_{zpc} ; i.e., charge reversal (Fig. 2a). In contrast, the net surface charge on NOM (Fig. 2b) is always negative as a function of pH. Both the relatively wide distribution of pK_a s and the coulom-

bic polyelectrolyte effect due to the presence of more than one ionizable surface group, contribute to the smeared aspect of the titration curve for humic substances, in which no clear inflection point can be distinguished (Morel and Hering, 1993). Titration curves for bacteria, which also possess heterogeneous surface functional groups, show the same smeared appearance as NOM (Fig. 2c shows the acid–base titration for the Gram-positive bacterium *Rhodococcus erythropolis* A177).

4.2.5. NOM attachment to solid surfaces

The concentration and reactivity of NOM in aquatic environments predicates its importance in a large suite of geochemical reactions. Specifically, the attachment of both dissolved and particulate NOM to mineral surfaces and NOM–metal interactions (Section 4.2.6) has been widely researched. Attachment of NOM to mineral surfaces: (1) results in size fractionation of NOM pool at the mineral surface, (2) appears to occur despite being energetically unfavorable, (3) changes the surface characteristics of the mineral surface irrevocably and non-additively, and (4) affects mineral–metal interactions. How NOM will affect metal–mineral interactions is dependent on the specific metal, the nature of the mineral surface and NOM molecules, and the NOM–mineral interactions. In some cases, the associated organic matter has been shown to increase metal scavenging while in others it has been shown to decrease metal sorption (see Section 4.4.1).

4.2.5.1. Size fractionation. Fractionation of NOM as a function of molecular weight has been observed at solid–liquid interfaces. This fractionation is both size and lability dependent. Higher molecular weight HA are preferentially sorbed to mineral phases compared to NOM particles, which are less aromatic and have a lower molecular weight (Davis and Gloor, 1981; Stumm, 1992; Zachara et al., 1994; Gu et al., 1995; Vermeer and Koopal, 1998; Lenhart and Honeyman, 1999; Meier et al., 1999). Chin et al. (1994) found a strong correlation between molar adsorptivity (gives degree of aromaticity, source functions, extent of humification and possible molecular weight), total aromaticity, and the weight average molecular weight for humic substances.

4.2.5.2. *Energy considerations for the adsorption of NOM to mineral surfaces.* There are several proposed mechanisms for NOM adsorption to mineral surfaces, including: ligand exchange, cation bridging, proton exchange, water bridging, hydrogen bonding and van der Waal interactions (Santschi et al., 1997; Lenhart and Honeyman, 1999). However,

none of these processes have been identified as the dominant mechanism. Schindler (1990) proposes that the adsorption is a result of three types of interactions: (1) hydrophobic expulsion, (2) electrostatic interaction, and (3) surface complexation. Hydrophobic expulsion is an energetically favorable process for molecules that have a greater affinity for the sorbent than the solvent in a given system.

The principles governing the energy associated with the adsorption of NOM to mineral surfaces are those that govern all adsorption reactions. Adsorption will occur spontaneously under the given conditions if the change in Gibbs free energy of reaction is negative. NOM sorbs to solid surfaces despite two unfavourable energy terms. A negative entropy term is associated with the adsorption, as the translational freedom of the NOM molecule is decreased. This negative term must be overcompensated by a negative enthalpy term (Atkins, 1994). However, the enthalpy of adsorption actually becomes less negative if the adsorbed molecules repel each other. This would be the case if humic acid were being adsorbed to a mineral under pH conditions above the pH_{zpc} of that mineral. Both the mineral and the humic acid are, under those conditions, negatively charged and sorption becomes even less favorable due to charge repulsion (Atkins, 1994; Au et al., 1999). Yet in spite of these considerations, NOM is observed to

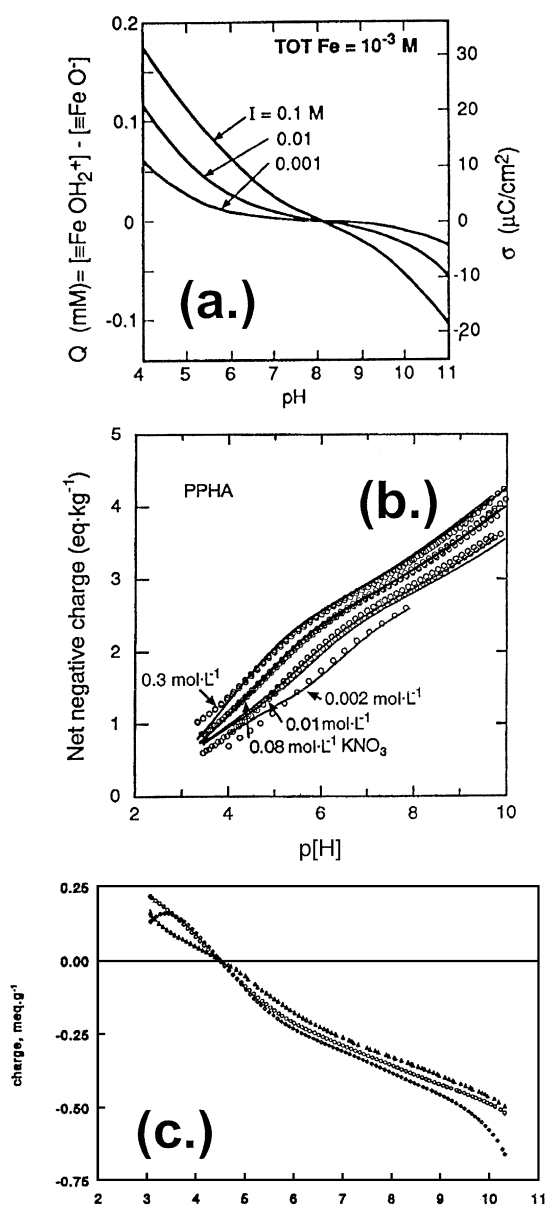


Fig. 2. Surface charge (net surface charge, Q ; and surface charge density, σ) as a function of pH and ionic strength (1:1 electrolyte) for hydrous ferric oxide (HFO) (a) (adapted from Stumm, 1992, with permission*); purified peat humic acid (HA), (b) (adapted from Kinniburgh et al., 1998, with permission*); and bacterial cell wall surfaces (isolated cell walls from *R. erythropolis*), (c) (adapted from Plette et al. 1995, with permission*). The absolute value of surface charge increases in an ionic strength-dependent manner, for all three surfaces. For the HFO surface (a), ionic strength-dependent surface charge–pH curves converge at the zero point of charge of the mineral HFO surface (above the pH_{zpc} of HFO, the mineral surface will be net negatively charged, while below the pH_{zpc} the HFO surface will be net positively charged). Both the bacterial cell walls (c), ($pH \sim 4.5$) and the HFO (a) ($\sim pH 8$) surfaces show the pH_{zpc} within the titration range of the experiments. In contrast, the pH_{zpc} of the HA surface is not accessible within the pH range of the experiments (b) ($\sim pH 3$ –10). The solid lines for the HA (b) are model fits (spherical double layer model; see text).

adsorb to metal oxide surfaces over a broad pH range. While, the adsorption of negatively charged HA to the positively charged hematite surface can be attributed principally to electrostatic attraction, adsorption of organic polyelectrolytes to negatively charged minerals cannot simply be electrostatic in nature. It is suggested that hydrophobic expulsion cause humic and fulvic acids to accumulate at mineral surfaces (Stumm, 1992; Au et al., 1999).

Lyklema (1985) postulates that there is an interaction-free energy involved in the adsorption of NOM to mineral surfaces. This energy is a result of the contact between specific segments of the molecule and the surface. The more segments in contact with the surface, the higher this energy term. As such, high molecular weight molecules may have an affinity for the surface that surpasses the unfavorable energy terms. The hydroxyl, phosphoryl and carboxyl groups that characterize NOM molecules are all groups that can effectively adsorb to surface sites (Stumm, 1992); i.e., they act as the segments that bind the molecule to the surface. Evanko and Dzombak (1998) reported that NOM sorption to goethite was best represented by compounds that have adjacent phenolic groups, adjacent carboxylic groups and highly acidic carboxylic acid groups ($pK_a \leq 1.5$), such as pyromellitic acid.

To picture how the energy barriers for NOM adsorption to mineral surfaces are overcome, it is useful to consider the adsorbed conformation of the humic substance. The traditional view of the adsorbed molecule is that it exists as a long chain made up of segments. “Train” segments are those segments adsorbed to the mineral surface, which are aligned parallel to the surface. “Tail” segments are the end segments of the molecule, which are oriented away from the surface and protrude into solution. “Loop” sections, segments between two adsorbed “train” segments, also extend into solution (Stumm and Morgan, 1996; Vermeer et al., 1997). The structural conformation of HA and FA change in solutions of different pH and ionic strength (Buffle, 1990), and thus, their adsorbed conformations are also predicted to be affected by the same parameters. It has been verified experimentally that at neutral and alkaline pH, organic macromolecules have an extended shape due to intramolecular repulsion (Stumm, 1992; Vermeer et al., 1998; Au et al.,

1999). Once adsorbed, such molecules form a flat layer on the surface of the mineral. In contrast, at low pH or high ionic strength, the molecules show a coiled conformation that when adsorbed, extend further from the surface of the mineral into solution.

In situ investigation of hydrated HA sorbed to mineral surfaces (muscovite) has recently become possible by atomic force microscopy (AFM). Supporting the experimental findings outlined above, Plaschke et al. (1999a,b) demonstrated that the adsorbed conformation of HA was dependent on solution pH. The pH-dependent structures were described as chains and aggregates (pH 4–5), particle-like/flat structure (pH 7.0), and, particles and torus/ring-shaped assemblies (pH 8). The average height and diameter range of the adsorbed structures increased with decreasing pH. In contrast, Maurice and Namjesnik-Dejanovic (1999) found that adsorbed peat FA formed highly porous, aggregate ring-like structures at pH ~ 5 (Fig. 3). Together, these results indicate that the adsorbed conformation of NOM molecules is dependent on solution pH and suggest that it is also dependent on the nature of the molecule (i.e., humic versus fulvic acid). The conformation of the ad-

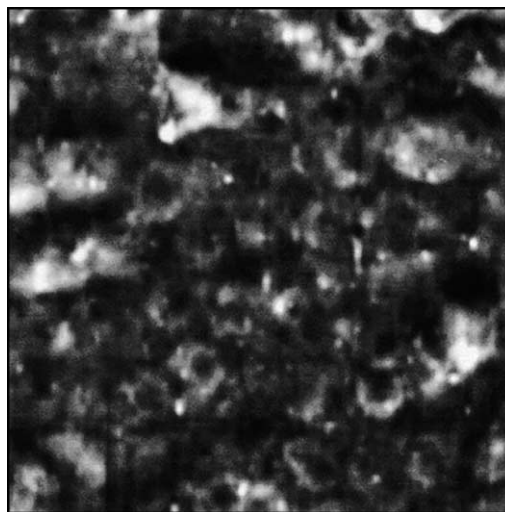


Fig. 3. Amplitude mode tapping mode atomic force microscopy (TMAFM) image taken in solution of a high molecular weight humic substance sorbed onto muscovite at pH ~ 5: $1.4 \times 1.4 \mu\text{m}$ from Maurice and Namjesnik-Dejanovic (1999), with permission*. Numerous ring structures of NOM on the mineral surface are apparent.

sorbed NOM molecule can potentially affect the reactivity of the mineral surface; the flatter the molecule to the surface, the greater the number of mineral–organic interactions, which may inhibit metal binding to the surface. The effects of NOM on metal binding to mineral surfaces is discussed in more detail subsequently (Section 4.4.1).

4.2.5.3. Effect of adsorbed NOM on mineral surface charge, charge density and potential. A reversal of surface charge of aquatic mineral particles due to NOM coatings is well established, irrespective of the nature and initial charge of the particle (Buffle, 1990; Fairhurst et al., 1995). For example, Au et al. (1999) showed that the charge of a hematite surface was profoundly affected by the adsorption of HA. The composite hematite–NOM surface had an overall negative charge at all pH values examined, indicating that the adsorption of the humic acid to the hematite caused a reversal of the surface charge of the mineral surface at pH below 8.1 (pH_{zpc} for hematite), and enhanced the negative charge of the surface at higher pH values. Adsorption of weakly acidic polyelectrolytes has also been shown to decrease the electric potential of a charged metal oxide surface by a value of up to 60 mV, depending on the extent of NOM adsorption and solution pH (Vermeer et al., 1998, 1999). The maximal change in surface potential was found at intermediate pH values. Both a decrease in the electric potential and an enhanced negative charge on the mineral surface have important implications for metal sorption to humic-coated metal oxides in natural systems. Metal sorption would be enhanced at high pH values and would occur over a much broader pH range, including pH values well below the pH_{zpc} of the mineral.

4.2.6. NOM and metals

The ability of organic molecules to scavenge trace metals is well known (e.g., Lion et al., 1982; Perdue and Lytle, 1983; Buffle et al., 1984; Davis, 1984; Fish et al., 1986; Weber, 1988; Bendell-Young and Harvey, 1992; Tipping and Hurley, 1992; Tessier et al., 1996; Dupré et al., 1999; Sauvé et al., 2000), and is a result of their negative surface charge. While NOM structural heterogeneity will impart differing geochemical reactivity to individual NOM molecules, there are common characteristics in NOM sorption

of metals that appear across systems. In particular, NOM affinity for certain metals especially Pb and Cu, but also Zn and Ni, is well known (e.g., Frimmel et al., 1984; Hansen et al., 1990; Warren and Zimmerman, 1994b; Logan et al., 1997; Lin and Wang, 1998; Tipping et al., 1998; Gao et al., 1999; Takács et al., 1999). Competition studies have also indicated that some metals bind strongly to sites on NOM (e.g., Al and Fe) and actually block the binding of other metals (Takács et al., 1999). Metal sorption to NOM induces potential changes that affect both sorbent and sorbate. Specifically, the interactions of metals with NOM can change the speciation, solubility, and bioavailability of metals, as well as cause changes in the behaviour of the organic matter composing the NOM itself (Takács et al., 1999).

Metals sorbed by organic matter tend to be more tightly bound than those to oxyhydroxides of Fe reflecting the stronger bonds commonly formed with organic functional groups. For instance, high molecular weight fractions have a higher apparent surface potential than lower molecular weight fractions, indicating that large humic molecules may have an enhanced ability to bind metals (Green et al., 1992; see Section 5.5.3.2). This difference might lead to the supposition that the potential for release of metals from NOM given changes in system chemistry may be less than those associated with oxides. However, degradation of NOM may lead to release of associated metals back into solution. Further, whether NOM is comprised of HA or more labile material will have a direct influence on the sorptive capacity of that NOM pool. In a field study of a sewage contaminated urban river, Warren and Zimmerman (1994a) found that the more labile organic matter in that system did not bind metals as effectively as had been shown for more refractory HA in the literature; and that Fe and Mn oxyhydroxides bound relatively more metal than the NOM fraction.

4.3. Bacteria

Surfaces of bacteria are analogous to mineral surfaces in that they possess surface functional groups that can sorb cationic species such as metals. Analogous to organic matter, bacterial surfaces possess a heterogeneous variety of functional groups; the exact types and associated densities of which are species

dependent. Compounding this complexity is the potential effect of metabolism, which can also affect the surface characteristics of a particular bacterial strain. Live, metabolically active cells, live non-metabolically active cells and dead intact cell surfaces can all possess different surface reactivities (e.g., Parmar et al., 2000).

Pace (1997) reports that only about 5000 non-eukaryotic organisms have been fully described although even a small soil sample reveals the presence of billions of organisms of innumerable different types. Prokaryotes, or bacteria, are characterized, with few exceptions, by a cell wall exterior to the cytoplasmic membrane that gives the cell structure its shape and relative rigidity (Holt and Leadbetter, 1992; Nealson and Stahl, 1997). Prokaryotes are differentiated from eukaryotic cells by their lack of a membrane-bound nucleus. Prokaryotes have a simple cellular structure, potentially high metabolic rates, short generation times, and the largest surface area to volume ratio of any living organism. This surface area to volume ratio is critical as they rely on diffusion for both nutrient supply and waste removal (Beveridge, 1984, 1989; Holt and Leadbetter, 1992).

4.3.1. Prokaryotic metabolism: implications for geochemistry

Bacteria show an incredible versatility in metabolic processes and live in many physically and chemically extreme environments. Metabolic processes include aerobic respiration, nitrification and denitrification, methane oxidation, sulfur oxidation and sulfate reduction, Mn and Fe reduction and oxidation, and methanogenesis. They effectively utilize environmental redox pairs to derive energy and/or nutrients for their own metabolic needs; and for this reason accumulate at redox interfaces. Extracellular enzymes produced by bacteria modify nutrient sources, and the nutrients are brought into the cytoplasm against a concentration gradient by specific transport systems (Nealson and Stahl, 1997). Many bacteria are able to adapt to new environmental conditions through the synthesis of those enzymes and other cellular components needed for those particular conditions (Holt and Leadbetter, 1992). Because bacteria are able to use electron acceptors other than molecular oxygen for respiration, including carbonate, ferric iron, nitrate, sulfate and various

organic molecules (Holt and Leadbetter, 1992; Nealson and Stahl, 1997), they impact all elemental cycles important in metal behaviour.

Essentially, bacteria are the primary agents of geochemical change due to their high surface area to volume ratio, widespread and abundant distribution, potentially high metabolic and growth rates, physiological responsiveness, evolutionary adaptiveness, and their incredibly diverse enzymatic and nutritional possibilities (Madsen, 1998). These characteristics ensure their dominant role as the catalysts for element transfer among various compartments and the fundamental cycling of elements (e.g., C, S, Fe, P) in the biosphere (Madsen, 1998). As pointed out by Lovley and Anderson (2000), the bioremediation potential of bacteria for organic contaminants using their promotion of redox reactions is well exploited; however, the potential of bacteria as metal reductants has not been well explored. For instance, Fe(III) reducing microorganisms can also reduce Tc(VII) to its insoluble (V) oxidation state as well as toxic Cr(VI) to the less toxic and soluble (III) oxidation state.

4.3.2. Surface reactivity of bacterial cells

To begin to understand the role of bacteria as reactive surfaces in aquatic systems, the chemical composition and structure of their cell walls must be described. Doyle et al. (1980) indicated that by that date bacterial surfaces were known to be amphoteric, to have the ability to bind metals, and to expand and contract with changing solution ionic strength. Further, it was later established that under most environmental pH conditions, the bacterial cell surface was known to be anionic as a result of negatively charged functional groups in the cell wall (Ferris and Beveridge, 1985).

4.3.3. Cell wall composition

The Gram stain, a differential staining technique for light microscopy, divides bacteria into two groups, Gram negative and Gram positive, on the basis of fundamental differences in their cell wall structural organization and chemical composition (Ferris and Beveridge, 1985; Nealson and Stahl, 1997; Urrutia, 1997). This method is, however, not absolutely reliable. The Gram-positive cell, exempli-

fied by *Bacillus subtilis* (Fortin et al., 1997) has a cell wall thickness of 20–50 nm (Beveridge, 1981, see Fig. 4). A Gram-positive cell wall comprises approximately 25 layers of cross-linked linear polymers of peptidoglycan (PG) (Beveridge, 1984). PG chains are made up of 40–50 repeating dimers of *N*-acetylglucosamine and *N*-acetylmuramic acid (Beveridge, 1984; Urrutia, 1997), and as such are rich in carboxylate groups (Fortin et al., 1997). Intermeshed in the PG framework is one of several secondary polymers, e.g., teichoic and teichuronic acids, proteins or sometimes lipids (Urrutia, 1997). Teichoic and teichuronic acids (TA and TUA, respectively) occur only in Gram-positive cells (Holt and Leadbetter, 1992), and can make up 50–60% of the mass of the cell walls (Beveridge and Murray, 1980). The secondary polymers TA and TUA differ in that TA contains phosphate groups and TUA does not. It has

been suggested that TUA is synthesized by bacteria growing under phosphate-limited conditions (Beveridge, 1984). Both the PG and the secondary polymers contribute to the overall anionic character of the Gram-positive cell wall, due to the pH-dependent deprotonation of carboxyl and phosphoryl groups.

The cell walls of Gram-negative bacteria are more complex than those of Gram-positive bacteria (see Fig. 4). They have a multilayered structure; a thin PG layer (without associated secondary polymers) is found between the chemically distinct outer and inner plasma membranes, as exemplified by *Escherichia coli* (Beveridge, 1984; Urrutia, 1997; Fortin et al., 1997). The PG and outer-membrane are attached by lipoproteins (Holt and Leadbetter, 1992) and together they constitute the cell wall of Gram-negative bacteria. The inner leaflet of the outer membrane is made up of phospholipid, the outer

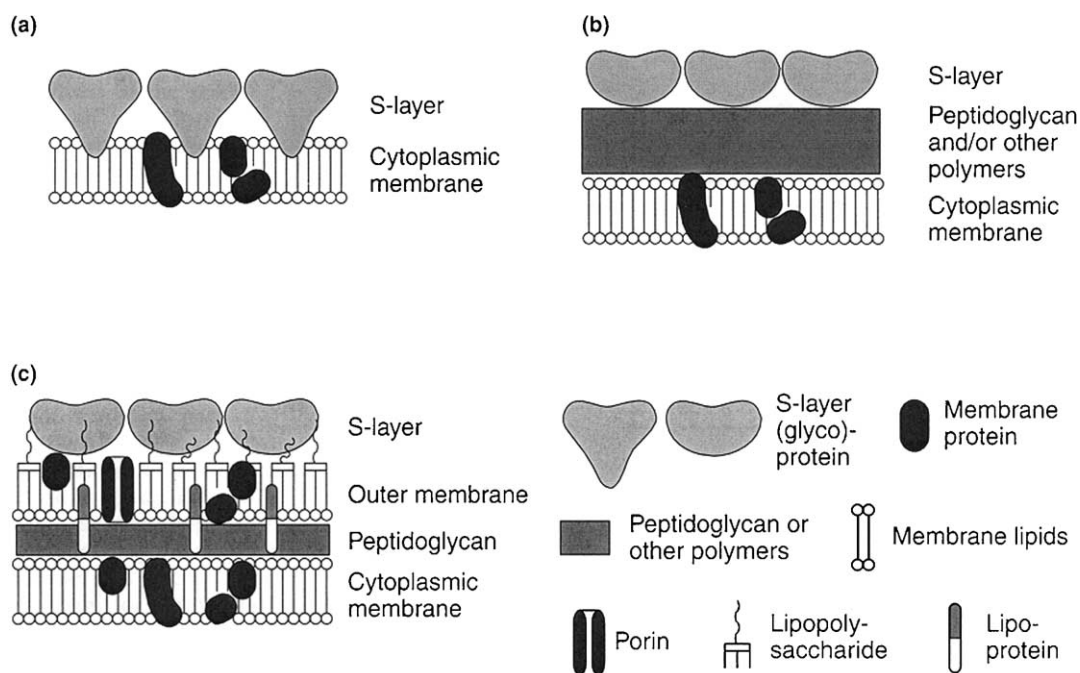


Fig. 4. Schematic illustration of the supramolecular architecture of the three major classes of prokaryotic cell envelopes containing crystalline bacterial cell surface layers (S-layers). (a) Cell-envelope structure of Gram-negative Archaea with S-layers as the only cell wall component external to the plasma (cytoplasmic) membrane. (b) The cell-envelope as observed in Gram-positive Archaea and Eubacteria. In Eubacteria, the rigid wall component is primarily composed of peptidoglycan. In Archaea, other wall polymers, (e.g., pseudomurein or methanochondroitin) are found. (c) Cell-envelope profile of Gram-negative Eubacteria composed of a thin peptidoglycan layer and an outer membrane. If present, the S-layer is closely associated with the lipopolysaccharide of the outer membrane (adapted from Sleytr and Beveridge, 1999, with permission^{*}).

leaflet of lipopolysaccharide (LPS). LPS is an amphiphatic molecule, made up of repeating units of oligosaccharides. The LPS is oriented such that the hydrophilic segments are exposed to the external environment. The phosphoryl groups from the inner leaflet and the phospholipids and carboxyl groups from LPS are responsible for the overall anionic character of the Gram-negative cell wall (Schiewer and Volesky, 2000).

4.3.3.1. Extracellular polymers and S-layers. Capsules, slimes and crystalline S-layers are highly hydrated (95% water) fibrous matrices that may be found in association with or surrounding various species of both Gram-positive and Gram-negative bacteria (Ferris and Beveridge, 1985; Urrutia, 1997). Capsules, or extracellular polysaccharides (EPS), are hydrophobic homopolymers or heteropolymers of carbohydrates and proteins (Urrutia, 1997). They constitute the most common of superficial layers (Urrutia, 1997). Capsules are generally tightly associated to the cell walls (Costerton et al., 1981; Holt and Leadbetter, 1992), and can reach a thickness of 3 μm around individual cells (Ferris and Beveridge, 1985). Slime layers have the same chemical makeup as capsules, but are not attached directly to the cell wall; they have been shed to the environment (Costerton et al., 1981; Holt and Leadbetter, 1992; Urrutia, 1997). The highly hydrated structures of both slime layers and capsules prevents the penetration of hydrophobic molecules through the cell wall and their anionic nature allows them to function as ion exchange resins (Ferris and Beveridge, 1985). Further, the long axes of EPS structures have been observed to extend perpendicularly from the surface of the cell, resulting in an extension of the negative charge of the bacterium beyond its negatively charged cell wall (Urrutia, 1997). This extension of negative charge from the bacterial surfaces has important implications for metal binding.

S-layers are highly structured and have been observed immediately external and in intimate contact with the cell walls of many bacteria and Archaea. They are single- or multiple-layered paracrystalline arrays of either protein or glycoprotein with linear, square, tetragonal, or hexagonal packing formats (Ferris and Beveridge, 1985; Holt and Leadbetter, 1992; Urrutia, 1997). The S-layer proteins fold dur-

ing self-assembly such that the polar amino acids are usually internalized and the external layer is more hydrophobic than the underlying cell (Fortin et al., 1997). The structure and arrangement of the S-layer of *Sporosarcina ureae* indicates that it may in part function as a molecular sieve at the surface by controlling inward and outward diffusion of large hydrophobic molecules (Ferris and Beveridge, 1985).

It is generally thought that the purpose of both capsules and S-layers is to protect the bacteria from the external environment, against changes in pH and increased temperature, as well as to assist in the adhesion of bacteria to other bacteria and surfaces (Ferris and Beveridge, 1985; Marshall et al., 1989; Holt and Leadbetter, 1992; Sigg et al., 1997). It is known that certain metals can induce the formation of capsular material (Appana and Preston, 1987), perhaps as a mechanism to reduce metal toxicity. For example, Beveridge (1984) reported bacteria that produce S-layers were able to survive in the presence of toxic metals. The metals were bound to the S-layers, which were sloughed off the bacterial surface into solution, and a new S-layer was produced. S-layers are often found in association with bacteria surviving in extreme conditions (Ferris and Beveridge, 1985).

4.3.4. Metal binding to bacterial cell walls, exopolymers and S-layers

A bacterium's potential sorptive capacity and affinity for various metals is dependent on the types and densities of functional groups associated with the cell. The types and densities of functional groups are, in turn, affected by the microbe's cell wall composition (Gram positive versus Gram negative), associated EPS or S-layers, and metabolic state. Further, bacterial surfaces also show a heterogeneous charge distribution; surface associations of metals are often localized at certain points. There is a growing interest in the use of bacteria as biosorbents for removal of metals from solution as their effectiveness as solid phase sorbents becomes increasingly evident (e.g., Volesky, 1990; Volesky and Holan, 1995; Schiewer and Volesky, 2000).

The overall anionic nature of bacterial cells in most aquatic environments is a result of the pK_a s of the carboxyl and phosphoryl groups (4–6 and ~ 7 , respectively) that, in different densities, make up the

cell walls of both Gram-positive and Gram-negative bacteria. Cell surfaces also possess amino groups, which at circumneutral pH are still positively charged ($pK_a \sim 9$). The relative abundance of these three groups in the cell wall as well as the metal-binding nature of each group will determine the metal-binding capacity of the cell surface at a given pH. Different surface sites may play a larger role in aquatic environments of low pH versus those at high pH.

Early research on metal binding to bacterial cell walls was focused on reactions occurring specifically at *B. subtilis* (Gram positive) cell surfaces (45% PG, 54% TA; Beveridge, 1984). Beveridge and Murray (1980) found that isolated walls of *B. subtilis* complexed substantial amounts of Mg^{2+} , Fe^{3+} , Cu^{2+} , Na^+ , K^+ , intermediate amounts of Mn^{2+} , Zn^{2+} , Au^{3+} , Ni^{2+} , small amounts of Hg^{2+} , Sr^{2+} , Pb^{2+} and Ag^+ , and did not complex Li^+ , Ba^{2+} , Co^{2+} and Al^{3+} at all. Further, some metals were bound more strongly than others. The metal complexing ability of the cell wall material was later attributed primarily to the carboxyl groups of the peptidoglycans (Beveridge, 1978; Doyle et al., 1980). Chemical modification of the carboxyl and amino groups of the *B. subtilis* cell wall established the importance of both groups for metal interactions with the surface, with an increase in metal-binding capacity was observed with the loss of amino groups (Doyle et al., 1980). Early research comparing the surface reactivity of *B. subtilis* with two other bacterial species, *B. licheniformis* (Gram positive) and *E. coli* (Gram negative), was summarized by Beveridge (1984). *B. licheniformis* cells walls contain less than half the PG of *B. subtilis* cell walls. In contrast to *B. subtilis*, it was separation of the TA and TUA from the PG of the *B. licheniformis* cell wall that resulted in a loss of the majority of the binding capacity of the wall for that species. *E. coli* has thinner PG layers and thus lower carboxylic site densities than both the two Gram-positive *Bacillus* spp. As a result, the *E. coli* cell walls bound the least metal.

More recently, differential metal affinity was noted by Puranik and Paknikar (1999). They reported that when Gram-negative *Citrobacter* strain MCM B-181 was exposed to multi-metal solutions, the metals were adsorbed preferentially in the order $Co^{2+} < Ni^{2+} < Cd^{2+} < Cu^{2+} < Zn^{2+} < Pb^{2+}$. In a re-

cent EPR study, Philip et al. (2000) showed that Cu(II) binding to *B. polymyxa* surfaces occurred by complex formation with oxygen and nitrogen atoms. Consistent with the earlier work suggesting the importance of carboxylic groups, this result inferred that Cu is likely bound by the carboxyl groups of the peptidoglycan layer and amino sugars or structural proteins.

As stated earlier, cell surfaces do not have an even distribution of charge density: pole-cylinder junctions and septa show a greater charge density (Urrutia, 1997). Correspondingly, metal deposition has been shown to occur non-uniformly on the surface of *E. coli*; metal binding is preferentially localized at the polar head group regions of the constituent membranes and along the PG layer (Mann, 1990). The important difference between the pH-dependent negative charge of bacterial surfaces and metal oxide surfaces or NOM, is that bacteria are living organisms and have some control over their surface charge due to metabolic processes and growth cycles (Urrutia, 1997). The metal binding capacity of the *B. subtilis* cell wall is decreased during active cell metabolism as a result of protons being pumped to the cell surface, effectively decreasing the number of available metal-binding sites. Isolated cell walls often have higher sorptive capacities than whole cells and may also possess different reactivities (Urrutia, 1997); most probably due to the exposure of more functional groups associated with cell decay (Ferris et al., 1988). Urrutia (1997) suggests that, as a result of the overall variability in chemical composition of cell wall structures and the difficulty in estimating the overall charge of the surface from isolated cell wall components, the best way to determine the total bacterial surface charge is through electrokinetic studies.

The reactions occurring at bacterial surfaces share the same characteristics as those occurring at mineral surfaces. One, all surface functional groups can play an important role in proton and metal binding to cell walls. Two, binding affinities and bond strengths vary among metals for the same surface sites. For bacteria, however, surface reactivity is made more complex in that the relative importance and density of the functional groups differs not only between Gram-negative and Gram-positive bacteria, but also among bacterial species. This last point underscores

the necessity for quantitative determination of the chemical nature of the sites and site densities associated with a variety of bacterial strains, under varying metabolic states. An important caveat to also consider for metal reactions occurring at bacterial surfaces is that bacteria in natural environments often co-exist in mutually beneficial, highly functionally structured consortia. The overall geochemical reactivity of such consortia is likely to be considerably different than those of the individual bacterial strains.

4.3.5. Microbial modes of existence: implications for metal geochemistry

There are several possible modes of bacterial existence in aquatic environments: planktonic, biofilms and suspended aggregate-flocs. The existence, dominance and importance of each mode varies among aquatic systems as well as within various compartments (e.g., water column, sediments, pelagic versus littoral) in any given aquatic environment; as well as for a given bacterial strain under different environmental conditions. Typically, cell densities in the pelagic zone are in the order of 10^6 /ml; in biofilms, they can reach 10^9 – 10^{11} /ml (Characklis, 1990). Thus, another factor that must be considered in the potential for bacterial scavenging of metals in aquatic environments is their dynamic, heterogeneous, non-uniform spatial distributions and concentrations. Bacterial influence can be relatively minor when considered for a system as a whole, but may play the dominant role in certain locales due to high cell density and associated activity.

Photosynthetic bacteria (commonly referred to in the limnological literature as blue green algae) must remain in the lighted or euphotic zone of a lake—either associated with the sediments in the littoral zone or free floating in the epilimnion. Bacteria that decompose organic matter, e.g., coupled to the reduction of sulphate or Fe(III) or Mn(III/IV) oxyhydroxides, are more commonly found in anoxic sediments. Biofilms represent a macroscopic “uber-bacteria”. To give the perspective on the sheer numbers of bacteria and relative potential for intensity of geochemical effect: the average size of bacteria is approximately 1–2 μm in length. Thus, it would take approximately 1000 cells lined up end to end to cover a distance of 1 mm. Bacterial biofilms can extend in physical size up to metres. These biologi-

cal structures comprising live and dead cells and organic matrices, represent a highly complex and highly structured environment where the macro structure of the biofilm, principally created by EPS secretion (Neu and Lawrence, 1997; Lawrence et al., 1998) helps irrigate the biofilm providing nutrients and removing waste products. Within the physical structure of the biofilm, metabolic function separates the various microbial inhabitants in tightly linked sequential reaction pathways from the biofilm surface to its bottom layer (Fig. 5; Canfield and Des Marais, 1993; Little et al., 1997). Thus, biofilms are biological reactors creating geochemical microenvironments that can differ substantially from the overlying water column. Further, the conditions occurring with biofilms are likely to be highly dynamic reflecting both the diurnal nature of biological activities as well as the natural aging process that occurs in biofilms or mats. Thus, metal dynamics within biofilms are likely to be substantially different from either the sedimentary or water column compartments; and their impact on bulk system geochemistry may be substantial. Further, these localized zones of potentially intense rates of geochemical reaction are not homogeneously spread throughout aquatic systems; rather, they occur in zones favourable to microbiological growth and survival. However, the extent of their relative influence on shaping metal cycling as a whole within systems, remains largely unknown.

4.3.5.1. Biofilms and metals. Metal sorption within biofilms or microbial mats has been shown to be highly effective. Early investigation of metal capture by biofilms was carried out by Ferris et al. (1989). Aqueous and biofilm-associated metal concentrations from an acidic (pH 3.1) and a circumneutral lake were quantified. Total biofilm associated metal concentrations exceeded aqueous metal concentrations in both systems. In the circumneutral lake, metal uptake and adsorption strength values were enhanced, with increases in metal uptake as great as 12 orders of magnitude, compared to the acidic system. Iron oxide precipitates associated with biofilm EPS were identified as ferrihydrite in both systems but the precipitate morphology and minor elemental composition was observed to vary between the two systems (Ferris et al., 1989). Minerals formed in

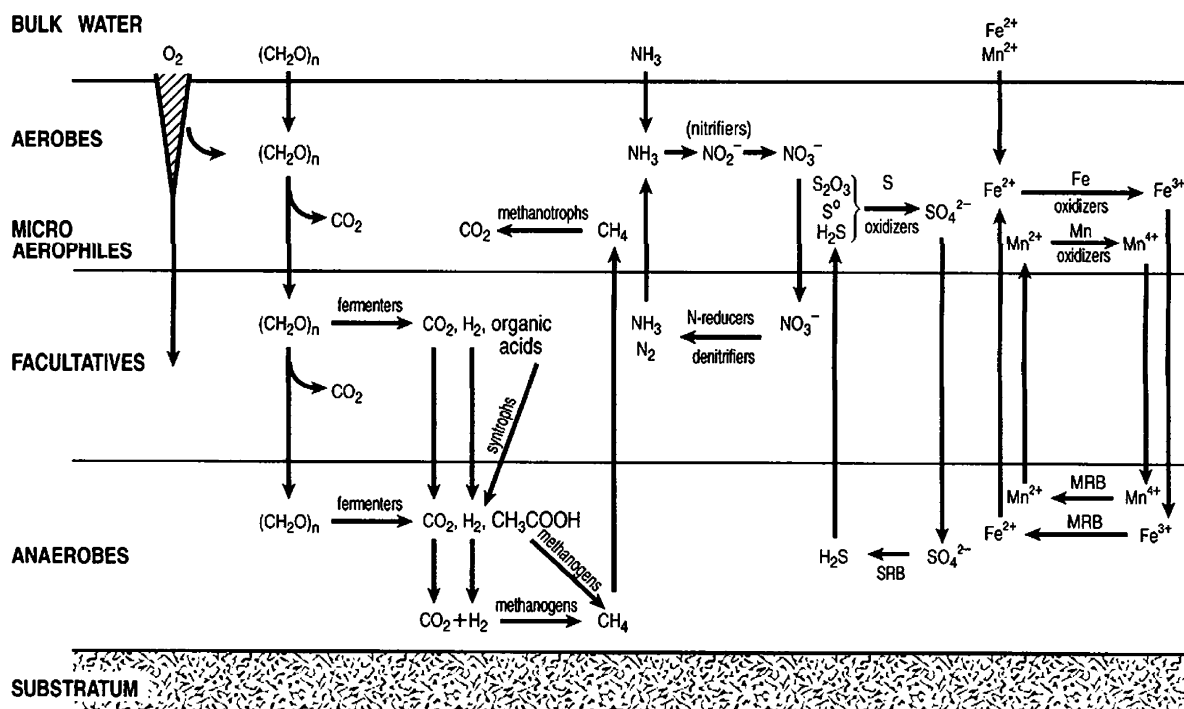


Fig. 5. Biofilm microbial stratification and resulting geochemical zonation within typical biofilm consortia (adapted from Little et al. 1997, with permission*). Microbial function is spatially separated within the biofilm leading to depth-dependent differences in geochemical reactions and conditions within the biofilm.

association with biofilms are commonly referred to as biominerals and are discussed subsequently in more detail (Section 6.1). Later, TEM investigation of a biofilm consortia from Canadian Shield groundwater showed that the accumulation of iron on the surfaces of cells was stepwise and that iron precipitates were found at the surface of the cells and throughout the biofilm matrix (Brown et al., 1998). Cell lysis was observed in some bacteria that had accumulated large amounts of iron precipitates at their surface. In contrast, bacteria that produced S-layers inhibited metal precipitation directly on the cell surface and remained viable.

Lünsdorf et al. (1997) observed typical *Siderocapsa*-like microcolonies in laboratory river water biofilms. The microcolonies that were typically made up of 6–30 cells surrounded by an outer envelope. Iron and manganese deposits were found exclusively at the surface of the microcolonies. In contrast, aluminum was detected not only at the microcolony

surface but penetrated the outer envelope and was detected within the intercellular space. Al was, however, not detected within the interior cytoplasmic space of the cells. The authors proposed that the EPS surrounding the cells immobilized Al, likely sheltering the cells against metal toxicity, and that the Fe and Mn were deposited at the microcolony surface through both active and passive processes and may act as energy sources for the cells. Similarly, Nelson et al. (1996) showed that Pb binding to EPS was three times higher than that observed to bacterial cells in a laboratory investigation of a pure culture biofilm of *Burkholderia cepacia*. The role of EPS in binding aqueous species was also investigated by Späth et al. (1998) who evaluated sorption of BTX (benzene, toluene, *m*-xylene), Cd, and Zn to the EPS and cell components of a biofilm. Although EPS is a highly hydrated structure, 60% of the lipophilic BTX was sorbed to the EPS matrix. In contrast, 80% of the total Cd content was sorbed to the cellular com-

ponent and increased Cd concentration resulted in an even smaller percentage of that metal bound to EPS. The same trend was found for Zn. These results indicate that the entire system should be considered when evaluating metal sorption, as organic contaminants can influence that behaviour.

Farag et al. (1998) showed that the greatest concentration of metals within foodweb components (biofilm, invertebrates, fish) from the mining waste contaminated Coeur d'Alene River Basin in Idaho, occurred in the biofilms. Schorer and Eisele (1997) showed that a river biofilm was enriched in Zn and Cu relative to sediment, but that Pb was not found in high quantities in the biofilm. The biofilm samples contained three times more organic matter than the sediment, and the different metal affinities of these two compartments was attributed to differences in the relative types and densities of different functional groups associated with the solid phases.

4.3.5.2. Metal capture—biofilm versus planktonic mode of growth. The mode of growth, planktonic versus biofilm, was shown to have a significant effect on metal binding by *Pseudomonas aeruginosa* PAO1 cells, but in a metal-dependent manner (Langley and Beveridge, 1999). Planktonic and biofilm forms bound equal amounts of Cu, but Fe, La and Au were bound to a greater extent by cells in the biofilm. Iron was observed to stain the whole cell of biofilm cells, but bound only to the surface of the planktonic cells. Larger crystalline deposits of Au were precipitated within the biofilm cells as compared to the planktonic cells; and apiculate La precipitates were observed to be associated specifically with surfaces of bacterial cells near the base of the biofilm structure. These differences were attributed to the different geochemical microclimate within the biofilm compared to the bulk, overlying water column.

4.4. Composite surfaces

Another complexity encountered in natural environments and one that must be addressed if accurate, representative models are to be developed, is the heterogeneity of natural solid matter. Single component, crystalline solids are rare if not non-existent in

natural environments; heterogeneous, multicomponent solids are the norm. The behaviour of such composite solids, e.g., oxide-organic complexes need to be evaluated and their surface chemistry quantified. The extent of composites and their effect on the metal scavenging abilities of the isolated reactive surfaces has only recently begun to gain attention (e.g., Small et al., 1999, 2000).

The components of a composite solid are all characterized by different surface properties (e.g., nature and densities of surface functional groups and complexing ability; Buffle, 1990). Many particles (organic, microorganisms and inorganic) in natural systems have surfaces that are coated by other reactive materials, including Fe(III) and Mn(III,IV) oxyhydroxides, and NOM. The surface properties of the coatings or secondary minerals are thought to be the dominant determinant for the behaviour of the composite solid, although they may only account for a minor fraction of the total composite mass (Buffle, 1990; Davis et al., 1998). The chemical conditions of a natural system are variable and will control the importance of biotic and abiotic components for metal sorption (Nelson et al., 1995). The extent of solid-phase partitioning of a given metallic element will depend on the affinity of that metal for the different components of the composite. In modelling the reactivity of these solids for various metals, the additivity rule is often employed. The additivity rule states that the extent of sorption by a composite solid will be the sum of the sorption due to its individual phases (Davies-Colley et al., 1984; Zachara et al., 1994). Implicit to the additivity rule is that the adsorption to each component can be described by a linear adsorption isotherm, and that the presence of one component does not affect the sorptive capacity of another (Zachara et al., 1994; Nelson et al., 1999b).

4.4.1. Mineral–NOM assemblages: the effect of NOM on metal binding to mineral surfaces

Generally, the extent of metal ion adsorption to mineral surfaces in the presence of NOM will be increased at low pH and decreased at high pH (Düker et al., 1995; Lenhart and Honeyman, 1999). Specifically, increased sorption to mineral surfaces in the presence of NOM at low pH (< 6) has been shown

for Co^{2+} (Zachara et al., 1994), U(VI) (Ho and Miller, 1985; Lenhart and Honeyman, 1999; Zuyi et al., 2000), Am (Moulin and Ouzonian, 1992), Cd^{2+} (Frimmel and Huber, 1996), Zn^{2+} and Yb^{3+} (Zuyi et al., 2000). In contrast, the presence of NOM was shown to decrease sorption of Cu^{2+} and Pb^{2+} to a variety of mineral surfaces (quartz, feldspar, kaolinite, calcite and Fe/Mn-coated quartz sand) at all pH values (Frimmel and Huber, 1996) reflecting most likely the higher affinity of these metals for dissolved organic compounds than for mineral surfaces. These authors, however, stress that the results could not be solely explained by metal-organic stabilities. A decrease in sorption in the presence of NOM was also demonstrated for Zn^{2+} specifically to quartz (Düker et al., 1995). However, these same authors note that the presence of NOM actually shifted the pH adsorption edge for Zn^{2+} sorption to both hydrazillite and goethite one pH unit lower. Warren and Zimmerman, (1994a) show that the presence of NOM in the suspended particulate pool of an alkaline urban river (pH \sim 8), increased Cu^{2+} sorption specifically to the Fe and Mn oxyhydroxide fraction of the suspended solid phase. However, the presence of NOM was not observed to affect the sorption of either Cd^{2+} or Zn^{2+} to the oxide fraction. Collectively, these results indicate that the presence of NOM in a system effects metal binding to mineral surfaces in a manner which is both metal and mineral specific. Moreover, the effect of NOM on metal sorption to metal oxides is not simply dependent on pH but is also dependent on: (i) the nature of the oxide; (ii) the nature of the humic substance; (iii) fractionation of the humic substance by sorption (discussed earlier); (iv) the relative strength of the metal-organic complexes, for both dissolved and sorbed NOM; (v) the extent of surface coverage by the humic substance; and (vii) the initial NOM concentration and the inorganic electrolyte concentration (Zuyi et al., 2000).

A further suite of studies investigated the effects of NOM adsorbed to mineral surfaces on mineral-metal interactions. Vermeer et al. (1999) evaluated the sorption of Cd^{2+} to hematite and specifically HA-coated hematite in batch experimental systems at high and low pH values and compared it to results by Robertson (1996) for sorption of Cu to hematite and HA-coated hematite. Their results showed that

the relative affinities of metals for each of the two components must be considered in the overall sorption to the composite. The overall sorption of a metal ion with a higher affinity for organic surfaces (e.g., Cu) will be lower due to masking of some organic sites by formation of bonds with the underlying oxide; while the overall sorption of a metal ion with a higher affinity for oxide surfaces (e.g., Cd) will be higher due to increased negativity of the surface charge due to the associated NOM. Fairhurst et al. (1995) found that HA specifically adsorbed onto a range of pure-phase inorganic colloids (δ -alumina, hematite, amorphous silica and titanium dioxide) increased the adsorption of ^{152}Eu at low pH and decreased adsorption at high pH, i.e., indicating that europium has a stronger affinity for organic groups. In contrast, the adsorption of ^{152}Eu to manganese dioxide was unaffected by the presence of HA even though the adsorption of HA to this colloid was shown to be significant at pH below 6 and to lower the electric potential (measured as zeta potential) of the mineral phase over the whole experimental pH range (2–10). These results indicate that manganese dioxide particles have an affinity for europium that is not affected by either an increasingly negative electric potential or by the adsorption of humic matter to the surface, which in theory could “block” potential metal-binding sites at the mineral surface. Zachara et al. (1994) reported that mineral-bound HA increased Co^{2+} sorption to subsurface mineral materials but appeared simply to augment, rather than change the intrinsic adsorption behaviour of the mineral sorbents. The authors proposed that the humic coatings form open, highly porous structures on the surface of the minerals. These structures, rather than impede or block metal-mineral complexation, would allow penetration of metals through the coatings to sites in the surface of the mineral. That such structures exist was confirmed by Maurice and Namjesnik-Dejanovic (1999) who reported that HA adsorbed at pH \sim 5 form highly porous ring-shaped aggregates with the mineral surface. The porosity of the aggregates would permit interaction between the mineral surface and bulk solution; for example, organic coatings may not hinder processes such as mineral dissolution. We suggest here, that these results may also explain the negligible effect of HA on the adsorption of some metals to specific mineral surfaces.

4.4.2. Mineral–bacterial composites

Studies examining the relative abilities of bacteria, minerals and bacterial–mineral composites to sorb metals are key to understanding how the nature of mixed solids can influence metal dynamics. Most of these studies to date, have been done in the laboratory (e.g., Walker et al., 1989; Flemming et al., 1990; Nelson et al., 1995; Small et al., 1999, 2000). However, Ferris et al. (1999) give comparative analyses of field based composite sorptive abilities and Nelson et al. (1999b) model Pb sorption to analogue composites based on characterized natural biofilms. A common result of the studies examining bacteria–Fe oxyhydroxide surfaces is non-additivity of the individual sorbents' sorptive behaviour in the composite. In general, the composite bacteria–Fe oxyhydroxide surface sorbs less metal than would be predicted by the sum of the individual component's sorptive capacities. A notable exception is the study by Nelson et al. (1995), which found that Pb sorption to biofilm Fe oxide composites was additive and predominantly associated with the Fe oxide component. This result is also contrary to the other mentioned studies where sorption of a variety of metals (including Pb; Walker et al., 1989; Flemming et al., 1990), was found to be greater to the bacterial component of the microbial–Fe oxide systems. Field studies examining the relative contributions of bacteria and minerals to metal sorption are very recent. Specific studies are discussed in more detail subsequently.

Early work on comparative metal (Ag, Cu, Ni, Cd, Pb, Zn, Cr) sorption by bacteria, clays and bacterial wall–clay composites done by Walker et al. (1989) and Flemming et al. (1990) indicated that on a dry weight basis, both Gram-positive (*B. subtilis*) and Gram-negative (*E. coli*) bacterial cell walls bound more metal than the clay components (kaolinite and smectite). The bacterial envelope–clay composite solids were observed to bind less metal (20–90%) than was predicted by the individual components' sorptive affinities. The amount of metal associated with the organic/cellular fraction of the composites was substantial, somewhere between 47% and 100% depending on the metal (Walker et al., 1989). Remobilization of the metals from the composites with the various agents (EDTA, Ca) was observed to fall between that seen for the individual

components, but not consistently so, indicating that these systems were complex and not predictable. For all samples, the metals were remobilized more easily from the clay components than from the organic component.

In contrast to this earlier work, Nelson et al. (1995) showed that Pb sorption to biofilm–Fe oxyhydroxide composites followed the additivity rule. These authors found that both planktonic and biofilm communities of *Pseudomonas cepacia* in a simulated flowing freshwater aquatic environment adsorbed Pb^{2+} . The planktonic sorption was insignificant at the cell density of the experiment (1% of the total lead in solution was adsorbed to suspended cells, cell density: 5×10^7 cells/ml). Within the biofilms, the presence of Fe oxyhydroxides was found to substantially increase Pb sorption to the biofilm (up to fivefold). The authors suggested that the presence of Fe oxyhydroxides buffers potential toxic effects of Pb on the bacteria as increased cell growth was observed in their presence.

Quantitative examination of the metal-binding capacities for each of two Gram-negative species (*Shewanella putrefaciens* and *S. alga*), amorphous hydrous ferric oxide (HFO), and bacteria–HFO composite solids were performed for the first time by Small et al. (1999, 2000). The bacteria–oxide composite was produced by allowing Fe(III) to first sorb to bacterial surface with subsequent promotion of amorphous iron oxide precipitation directly at the bacterial surface. In the first study, the sorption of Sr^{2+} to the individual components and composite was quantified using a generalized Langmuir equation. In a second study, the effects of ionic strength on the Sr^{2+} binding capabilities of *S. alga*, HFO and bacteria–HFO composite were examined. Finally, the group investigated Sr^{2+} sorption to isolated bacteria (*S. alga*), HFO and bacteria–HFO composites with varying proportions of HFO precipitated at the surface, ranging from 0.2 to 0.8 mass fraction HFO (unpublished data). The results demonstrated that the two bacterial species had surface complex formation constants and maximum binding capacities approximately two orders of magnitude greater than those measured for the HFO. Metal binding isotherms to the composite were observed to follow that of metal binding to isolated bacteria, but the maximum binding of the metal to the composite was 21% lower

than that calculated based on mass fraction of HFO and bacteria making up to composite. The study indicated that while the bacterial functional groups with low pK_a s remain important for the binding of Sr^{2+} to the composite, many of the binding sites appear to be “masked” by the HFO precipitated by association with the bacterial cell walls. Only those sites with highest apparent surface complex formation constants bound Sr^{2+} at high IS. HFO showed inner-sphere type sorption (i.e., adsorption) of Sr^{2+} . The composite, surprisingly, showed a greater sorptive capacity than both the isolated components combined at high IS. This effect was explained as a unique composite surface charge that buffered the effects of high ionic strength in terms of Sr^{2+} sorption. Overall, of all the surfaces studied, the isolated bacterial surface bound the most Sr^{2+} , and the pure HFO surface, the least. The extent of metal sorption to the composite solids was in all cases shown to follow directly from the proportion of bacteria in the composite. Distribution coefficient (K_D , solid phase metal:solution metal ratio) values measured as a function of HFO content of the composite showed a strong inverse relationship.

Ferris et al. (1999) quantified the metals bound to the bacterial iron precipitates (BIOS) in a biofilm from a subterranean site (pH 7.0–8.0) in Sweden. The microbial ecology of the biofilm was found to be quite heterogeneous. The lithoautotrophic, ferrous-oxidizing bacterium *Gallionella ferruginea* dominated the community, and *Caulobacter* spp. or *Hyphomicrobium* spp. was also identified. TEM, EDS and powder X-ray diffraction analyses of the BIOS specimens showed that the iron oxide precipitates had very little long-range crystallinity, and were not found exclusively associated with the bacterial cells but were found throughout the biofilm in association with EPS. Hydrated ferric oxide was found to account for 64–89% (dry weight basis) of the solid mass of the biofilm samples, with the remaining mass was speculated to be derived from bacterial organic matter. Total biofilm metal concentrations were quantified and found to be higher than their respective concentrations in the overlying water. The oxide fraction of the BIOS was shown to bind 50–100% of the biofilm associated metals. Na and Mn were found to be associated with the oxide fraction but Zn, Co and Cu showed affinity for the organic/residual

fraction. The most important trend shown by this study was the trend of decreasing K_D with increasing oxide content of the BIOS, and that this trend was shown for each metal, meaning that the amount of metal associated with the BIOS was related to the oxide/residual ratio.

Nelson et al. (1999b) examined lead sorption by natural biofilm samples from three lakes and a water-supply well. The mineral and organic components of the natural biofilm samples were identified using sequential extraction techniques. Pb sorption to whole biofilm samples was then measured in the laboratory over a range of Pb concentrations. The extent of Pb sorption was found to increase with increased Fe and COD (chemical oxygen demand; i.e., cell growth). Model analogs of the metal oxide and organic phases identified in the biofilm were used to estimate their relative contribution to the total Pb sorbed to the biofilm. Pb sorption to the organic phase was represented by sorption to pure cultures of *N. pelliculosa* (freshwater diatom), and *C. vulgaris* (green algae), *L. discophora* and *P. cepacia* exopolymer. Amorphous iron (III) oxides, biogenic Mn hydroxide and Al oxide were used to model the mineral phases of the biofilm. The final phase represented the remaining inorganic residue in the sample, after acid extraction of trace elements.

The following trends were observed for Pb sorption to the biofilm–mineral composites in the lake biofilm samples: the contribution from the Fe and Mn oxyhydroxides was greater than that predicted for the combined organic phases. The contribution of Mn oxyhydroxides was estimated to be greater than that of Fe oxyhydroxides, especially at low Pb concentrations. Overall, non-additivity occurred: more Pb was sorbed by the biofilm composites than was predicted. The authors postulate that this result may reflect differing reactivities of abiotic and biogenic Fe oxyhydroxides (the authors modelled sorption to abiotic Fe oxides), and other interaction of microorganisms with the surfaces of metal oxides including the formation of ternary complexes; or an unaccounted for sorbent in the composite.

4.4.3. Mineral–NOM–bacterial assemblages

Fein and Delea (1999) used EDTA, a synthetic analogue for NOM, to simulate the effect of natural organic ligands on the sorption of Cd^{2+} to the

surface of the bacteria, *B. subtilis*. Both (2,4,6-trichlorophenol) TCP (Daughney and Fein, 1998a) and EDTA were reported to sorb to the bacterial surface, with the maximum sorption occurring at pH values before their first pK_a values (6.0 and 1.9, respectively), and rapid decrease in sorption at higher pH values. The sorption behaviour of these two organic acids to the bacterial surface mirrors the sorption behaviour of NOM to metal oxide surfaces (as discussed earlier). Both the bacterial surface and the EDTA are negatively charged above pH 1.9 and become progressively more negatively charged with increasing pH, and therefore sorption becomes increasingly unfavorable due to electrostatic repulsion. The sorption of EDTA below pH 1.9 can be attributed to hydrophobic expulsion (Fein and Delea, 1999). The presence of EDTA in the system significantly decreased the extent of Cd^{2+} sorption to the bacterial surface. When the EDTA concentration was 10^{-3} – 10^{-2} molal, Cd^{2+} adsorption was insignificant even at high pH (7–8) where the bacterial surface charge is strongly negative.

Fein et al. (1999) followed up these preliminary studies with a comparative investigation of humic acid adsorption onto *B. subtilis*, Al-oxide mineral surfaces, and three mixed bacteria–mineral systems. HA was found to adsorb to the bacteria at low pH when both the bacterial cell wall and HA are uncharged, due to hydrophobic interaction. The modelled results implied that the adsorption of HA to the cell was governed specifically by the speciation of the carboxyl groups. The greatest extent of HA adsorption to the mineral surface occurred at pH values when the two particles were oppositely charged, as expected. Adsorption to the mixed systems was predicted reasonably well using the surface complexation model using only the results from the two-component (e.g., HA–bacteria) systems. HA was shown to be almost completely bound in conditions of pH 5.0–5.5 in the mixed systems, with the extent of adsorption negatively correlated to pH. However, the model underpredicted the adsorption of HA at pH 7.0–8.5, making it disadvantaged for modelling natural environments.

Collectively, these studies of mixed mineral–NOM, mineral–bacterial, NOM–bacterial and mineral–NOM–bacterial assemblages manifest the complexity of determining the processes that dominate

metal partitioning between phases in natural environments. Commonly, the organic fraction dominates metal binding in clay–bacteria and bacteria–HFO composites and metal–organic binding is stronger than those interactions between metals and minerals, even in the presence of strong remobilizing agents—but not always. Bacterial surfaces are often shown to sorb greater quantities of metals compared to Fe oxides (e.g., Kurek et al., 1982; Gonçalves et al., 1987; Xue et al., 1988; Small et al., 1999). However, a far greater quantitative understanding of mixed mineral–organic metal sorption dynamics is required for development of an accurate model of metal behaviour in natural systems.

5. Modelling approaches

Metal-sorbent dynamics are heterogeneous; reflecting specificity in both the metal(s) involved, as well as the solid surface(s), i.e., types and densities of surface functional groups are solid dependent as discussed previously. The complex reality of metal sorption reactions has lead to a variety of modelling approaches. The diversity of models reflects not only the geochemical diversity of surface reactions but also the varying complexity depending on surface type (e.g., simple mineral or NOM) and solution conditions (e.g., ionic strength).

All geochemically based modelling techniques have as their basis, those laws governing coordination reactions in solution; i.e., complexation reactions between ligands and (for our purposes) protons and metal ions, characterized as chemical equilibrium models. The complexities of modelling proton and metal ion binding to heterogeneous sorbents such as bacteria, metal oxides and natural organic matter are twofold: one, bacterial cell walls, metal oxide surfaces, and NOM molecules are charged so that long-range electrostatic interactions must be accounted for to describe the complexation reactions in a physically meaningful manner; and two, sorbent–sorbate interactions cannot be simply represented by a single type of binding site because of the heterogeneity of functional groups present on the sorbent. Rather, “binding sites” are created purely for modelling convenience and must not be mistaken either to directly represent the actual physical reality of the

surface functional groups or to permit interpretation of the surface reactions at the molecular level (Bufle, 1990).

Ideally, geochemical models should address all of the following considerations: (i) How do the electrostatic effects and the ionic strength of a solution affect complexation reactions at the solid–liquid interface? (ii) How penetrable are the sorbents to the sorbate species? (iii) Can we characterize competitive cation binding (e.g., proton–metal ion or metal ion–metal ion) to the same sorbent over a wide range of chemical conditions? (iv) How does time affect the establishment of equilibrium partitioning of the sorbent between the solid and liquid phases? In reality, the first two considerations have been, and continue to be extensively studied, the third consideration is a more recent development, and the last consideration is largely ignored. In this discussion, we present an overview of the various modelling approaches outlining how they can be classified and understood, how they address the complexities outlined above, how they relate to quantifying metal surface reactions, and further, how their applicability and utility is dependent on the natural system to be modelled.

5.1. Model classifications

Models can be grouped by five interrelated classifications: by model type: discrete versus continuous; by mathematical solution methods: analytical versus numerical; by approach: deterministic versus empirical; by sorbate: H^+ versus M^{z+} ; and by mechanism: simple ligand, pure surface, and polyelectrolyte. For the purpose of simplification, our description of the models will use protons as the sorbate, because the framework for most models began with the description of acid–base titration data, i.e., a description of net surface charge versus pH with addition of acid or base. Binding of metals ions and the resulting competitive binding of metals ions with H^+ were considered as an addition to the preexisting framework.

5.2. Model type

The model type addresses the complexity of representing the intrinsic heterogeneity of sorbents. Proton binding is thought to occur at a number of independent monoprotic sites (≥ 1) distributed ran-

domly on the macromolecular surface. All binding sites have associated equilibrium constants (K_a), expressed as pK_a ($-\log K_a$) values, which when plotted against their abundance (mol kg^{-1}), yields a pK /affinity spectrum (Luenberger and Schindler, 1986). There are two model types: discrete and continuous. Discrete and continuous models differ in their representation of the binding sites used to reproduce the experimental acid–base titration data. We use the term “binding site” as a collective term for surface binding sites or functional groups, which demonstrate chemical equivalency; i.e., a specific type of carboxylic or hydroxyl group. Discrete models fix the number of binding sites to a (usually small) finite number, and each site is considered to have a unique (or discrete) pK_a value. Overall proton binding to the sorbent is then mathematically described as the linear sum of Langmuir isotherms for each of the individual sites (Borkovec et al., 1996). Isotherms express a relationship between the bulk aqueous phase activity (concentration) of the sorbate and the amount sorbed at a constant temperature (Stumm and Morgan, 1996). Langmuir isotherms are the simplest of adsorption isotherms, assuming a 1:1 stoichiometry at the binding sites. Alternatively, proton binding curves can be mathematically described as a summation of monoprotic ligands (Brasard et al., 1990). In both cases, the solution is numerical rather than analytical, meaning that a set of pK_a values is found through an iterative procedure discussed briefly below (Section 5.3). The discrete model requires two adjustable model parameters for each binding site, the K_a value and site concentration. A discrete model pK spectrum, derived from the acid–base titration of an aquatic humus sample (Fig. 6a), is shown in Fig. 6b. The number of peaks in the distribution corresponds to the chosen number of unique binding sites. The number of sites that is chosen is somewhat arbitrary; it is often a choice between the minimum number of sites needed to give a good fit to the experimental data (thus, minimizing the number of model parameters) and the number of sites that the researcher feels gives a better estimation of all the possible sites, or of the functional groups of the sorbent (Cox et al., 1999).

The continuous model is based on the theory that the sorbent is comprised of an infinite number or

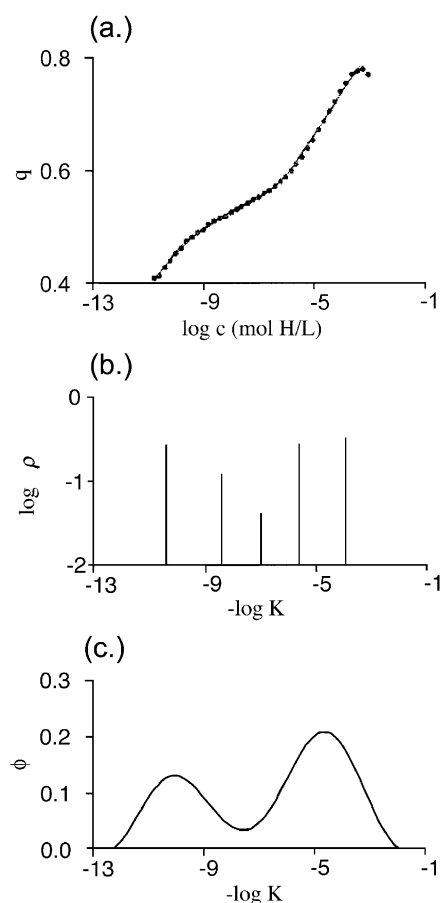


Fig. 6. (a) Acid–base titration of an aquatic humus as a function of pH ($\log c \text{ (mol H/L)}$). Note that the authors refer to this curve as a function of the common logarithm (basis 10) of the proton concentration. Discrete model (b) and continuous model (c), fit to the experimental titration data (adapted from Černík et al. 1995; with permission^{*}).

monoprotic sites with independent reactivities. Instead of describing overall proton binding as the linear sum of adsorption isotherms for each site, it is alternatively expressed as a continuous function of Langmuir isotherms. Not all these continuous equations can be solved analytically so that continuous models are often simplified to allow for a mathematical solution to the description of sorbent binding sites. Perdue and Lytle (1983) did so by combining the expression for a “local” (Langmuir) isotherm with a Gaussian distribution function, to approximate the experimental distribution about the local isotherm.

Using such a model, three fitting parameters must be optimized: the mean pK_a value (derived from a Langmuir isotherm); the standard deviation of the distribution about that pK_a value; and the total number of sites (Martinez et al., 1998). Several research groups have used Gaussian affinity distributions of pK values (Dzombak et al., 1986; Dobbs et al., 1989; Susetyo et al., 1990; Stevenson et al., 1993; Manunza et al., 1995). A disadvantage to the Gaussian distribution is that numerical integration is necessary to yield the overall isotherm (Kinniburgh et al., 1998). Alternatively, analytical binding equations can be used to describe proton-binding curves. Three such equations are the Generalized Freundlich (GF), Langmuir–Freundlich (LF), and Tóth isotherm equations. Each of the isotherm equations is associated with a monomodal distribution function. The distribution function associated with the LF equation, for example, is symmetrical and pseudo-Gaussian (Milne et al., 1995b). With regards to choosing an appropriate binding equation for modelling heterogeneous sorbents that may have multiple functional group types (e.g., carboxylic and phenolic for NOM), a Gaussian distribution is advantageous as it readily distinguishes monomodal and bimodal distributions (Perdue, 1985), whereas two of the LF, GF or Toth isotherm equations must be combined to give a bimodal distribution. All the distribution functions are disadvantaged in that they make a priori assumptions about the shape of the affinity distribution (Perdue, 1985).

It is important to keep in mind that model-derived binding sites do not directly represent the functional groups of the sorbent. The identity of the “binding sites” can only be inferred by comparison of the binding site pK_a values with pK_a values of model compounds (for example, carboxyl and phosphoryl groups for bacterial surfaces). Spectroscopic evidence is needed to link the modelled sites to “real” surface functional groups (Borkovec et al., 1998; discussed subsequently).

5.3. Solutions

In general, discrete models are associated with numerical solutions and continuous models with analytical solutions, although as indicated above, continuous models often require numerical solutions.

Numerical solutions involve fitting models to experimental data by optimization of suitable model parameters. Optimization techniques are iterative and involve minimizing an objective function, which is an equation that expresses the difference between the model predictions and the experimental measurements. The minimization process continues until the value of the objective function converges to a “global” minimum. Objective functions include classical non-linear least-squares methods (used in FITEQL, Herbelin and Westall, 1994), regularized least-squares methods (Černík et al., 1995), and the absolute sum of the error of fit (Brassard et al., 1990). Optimizations using classical least-squares methods are, however, “ill-posed”, as many affinity distributions result in a good fit to the experimental data (Buffle et al., 1990; Černík et al., 1995; Borkovec et al., 1996). Minimization of the objective function as the absolute sum of the error fit emphasizes differences among the concentrations of specific sites, whereas least-squares minimization de-emphasizes such differences (Smith and Kramer, 1999b). Using the first method, less abundant binding sites are included in the final solution.

5.3.1. Goodness of fit

The “goodness of fit” of a model to experimental data is a term used frequently, and expresses the degree of minimization of the objective function, i.e., how well, mathematically, the model fits the experimental data. As such, the “goodness of fit” cannot be considered a parameter of the success of the model to describe the true nature of the sorbate–sorber interaction, rather, it is simply a reflection of the model’s ability to optimize the adjustable parameters. This distinction is paramount in understanding the true value of modelling and its limitations. A model is simply the statement of a hypothesis in terms that can be compared with accessible data. An acceptable “fit” of the model simply means that the proposed hypothesis could explain the phenomenon. The main advantage of a model is thus to force its creator to be as consistent as possible.

5.4. Empirical versus deterministic

Deterministic models are heuristic, meaning they attempt to represent known mechanisms or hypothe-

ses within a system, and allow formulation of further hypotheses about that system. A deterministic model developed to describe the qualitative features of experimental sorption data includes both explicit chemical and electrostatic models. Deterministic models tend to describe a large set of solution conditions with acceptable accuracy. In contrast, empirical models rely on fitting the data to some convenient equation without regards to mechanisms, and as such, tend to accurately describe only a particular set of conditions, e.g., metal sorption for one environmental system. Semi-empirical models are those models that use “fudge factors” such as correction terms and power functions to reduce the number of fitting parameters needed to give a good description of the experimental data. Most models for heterogeneous sorbents are semi-empirical due to the innate complexity of the sorbent and because the interactions at the solid–solution interface cannot currently be visualized accurately.

5.5. Mechanism: simple ligand, pure surface and polyelectrolyte

Because metal oxide surfaces, bacterial cell walls and NOM are charged in aqueous environments, the most fundamental challenge for all models, is how to quantitatively represent the dynamically charged interfacial region that occurs between the solid and the bulk solution. Models attempt to account for electrostatic effects by employing a number of hypothetical physical representations that locate either specifically adsorbed ions and/or counter-ions in the interfacial region, and as such, quantify the sorbent/surface charge at a given pH.

5.5.1. Theoretical models of the interfacial region

A complexation reaction between a sorbent functional group (L) and a sorbate (M) at equilibrium is generally represented as



and the associated equilibrium constant for such a reaction is expressed

$$K_{ML} = [ML]/[M][L]. \quad (3)$$

In the case, where a sorbate ion is adsorbed to a charged sorbent, the total energy of adsorption is the sum of short-range chemical (intrinsic), and long-range electrostatic (coulombic) energy terms (Morel and Hering, 1993)

$$\Delta G_{\text{adsorption}} = \Delta G_{\text{intrinsic}} + \Delta G_{\text{coulombic}} \quad (4)$$

Eq. (4) is the basis of all adsorption models (Morel et al., 1981). The energy contribution of the electrostatic interactions corresponds to the work necessary to bring an aqueous ion, M^{z+} , from the bulk solution through the interfacial potential gradient, Ψ , to the reactive site on the sorbent (Buffle, 1990; Stumm, 1992).

$$-\Delta G_{\text{coul}} = \ln K_{\text{coul}}/RT = -\Delta Z(-F\Psi/RT), \quad (5)$$

where ΔZ is the net change in charge number due to the adsorption reaction, F the Faraday constant, R the ideal gas constant, T the absolute temperature. The “apparent” equilibrium constant for adsorption, is expressed

$$K_{\text{apparent}} = K_{\text{int}} \times K_{\text{coul}} = K_{\text{int}} \exp(-\Delta ZF\Psi/RT). \quad (6)$$

The exponential term in Eq. (6), $(-\Delta ZF\Psi/RT)$, is referred to as the Boltzmann factor and essentially expresses the effect of the interfacial potential at a charged surface. The subscript “int” used in the above expression is short for intrinsic. The intrinsic equilibrium constant, K_{int} , corresponds to K_{ML} from Eq. (3). Both are the equilibrium constants for the adsorption reaction at zero net surface charge (Goldberg, 1992), i.e., they express the purely chemical bond formed between sorbent and sorbate without an electrostatic component. Analogously, $[M]$ in Eq. (3) corresponds to the concentration of the sorbate, M , at the binding site, not in the bulk solution, i.e., the concentration of M accumulated in the interfacial region as affected by the electric potential of the charged sorbent

$$[M] = [M_{\text{bulk}}] \exp(-\Delta ZF\Psi/RT). \quad (7)$$

Eq. (8) applies if the sorbent in Eq. (7) is a proton ($\Delta Z = 1$)

$$\text{pH} = \text{pH}_{\text{bulk}} - 0.434(F\Psi/RT). \quad (8)$$

Because the interfacial potential gradient, Ψ , is not a directly measurable parameter, models of the reactions at the solid–liquid interface must rely on a “best guess” representation of the charge distribution as it occurs between the solid and bulk solution. Thus models that account explicitly for electrostatic effects must derive an expression relating potential to a measurable (or fitted) parameter.

All theoretical models of the interfacial region (1) relate the interfacial potential to the measured total sorbent charge, (2) involve some knowledge or assumptions about particle geometry (Dzombak and Morel, 1990; Milne et al., 1995b) and permeability, and (3) describe the accumulation of counter-ions in the interfacial region.

Total sorbent charge is the sum of four components: the permanent structural charge of a sorbent (e.g., isomorphic substitution in clay particles), the net proton charge, and the inner- and outer-sphere complex charges (Stumm, 1992).

The geometry and permeability ascribed to the sorbent govern the spatial distribution of the total sorbent charge. With regards to permeability, sorbents have been modelled as two-dimensional impenetrable surfaces or, alternatively, as three-dimensional permeable gel-phases. If a sorbent is described as a permeable gel-phase, the assumption is then made that the total sorbent charge is uniformly distributed throughout a given sorbent volume. The theoretical model used to describe the surface charge–potential relationship for such sorbents is the Donnan model. In this review, we refer to any modelling approach that uses the Donnan model as a polyelectrolyte model. This is because high molecular weight polyelectrolytes (namely HA) have classically been modelled as permeable phases using the concept of Donnan equilibrium. Bacteria, NOM and metal oxides have all been modelled both as polyelectrolytes and as pure surfaces. When a sorbent is modelled as a pure surface, the assumption is made that the total particle charge is distributed uniformly over a uniform surface. There are two common geometries used to model pure surfaces: infinite planes and spheres. For such sorbents, electric double layer (EDL) models yield the surface charge–potential relationship.

Both Donnan and EDL models assume an accumulation of counter-ions and expulsion of co-ions

from the interfacial region, although such assumptions need to be tested by direct measurements (Brown et al., 1999a,b). This accumulation of countercharge maintains electrical neutrality between the charged sorbent and the bulk solution.

Regardless of whether the sorbent is modelled as a pure surface or as a polyelectrolyte, Eq. (6) holds, meaning that the intrinsic equilibrium binding constants for metal and protons are accessible only if both the electrostatic and chemical components of binding are treated explicitly. Fig. 7 illustrates this

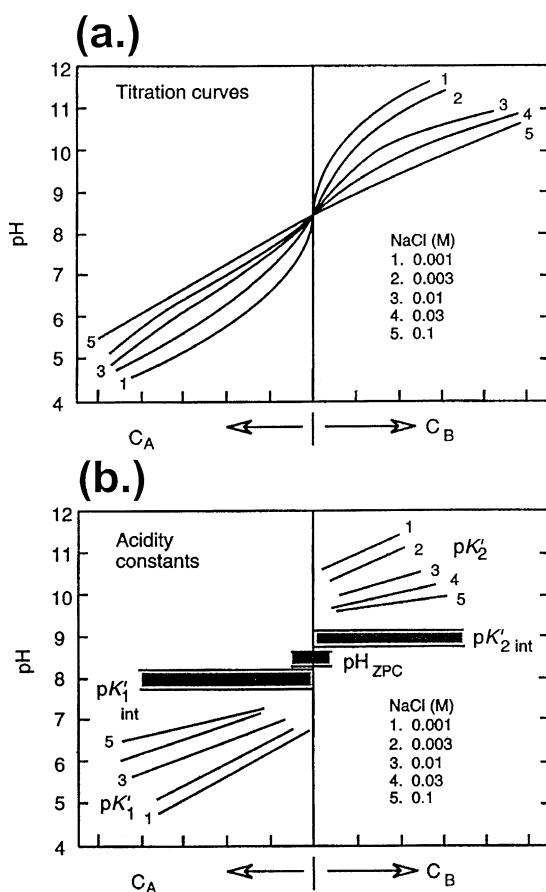


Fig. 7. (a) Titration curves in different concentrations of NaCl background electrolyte and corresponding acidity constants for hydrous $\gamma\text{-Al}_2\text{O}_3$. The uncorrected acidity constants (pK'_1 and pK'_2) are calculated from the corresponding individual points on the titration curves. They vary as a function of surface charge whereas the corresponding intrinsic constants (pK'_{1int} and pK'_{2int}) do not; they all plot in the shaded areas on the figure. The titration curves all intersect at the ZPC (zero point of charge; Drever, 1997, with permission*).

concept. In the upper panel (Fig. 7a), is the titration curve for hydrous $\gamma\text{-Al}_2\text{O}_3$ at different ionic strengths (C_A and C_B are the amount of acid and base added, respectively). The ionic strength effects on proton binding to this mineral surface are analogous to those effects shown for proton binding to hydrous ferric oxide (Fig. 2a). Evident again is the intersection of the titration curves at the point of zero charge, being the point at which the mineral surface is uncharged. Consider for the moment only the titration curve “1”, corresponding to an ionic strength of 0.001 M. Proton binding constants, expressed here as pK_a values, could be calculated from each point along this titration curve, as shown in Fig. 7b. The sloped pK_a lines marked “1” in this panel are so shaped because the binding of protons to this surface includes a coulombic term and as such, the pK_a values vary with changing surface charge. These pK_a s are “apparent” constants. In contrast, intrinsic equilibrium constants remain constant as surface charge is varied (shaded areas in Fig. 7b), indicating that the complexation between the metal and the sorbent does not include a coulombic term. Note that as the surface charge approaches zero, the apparent pK_a lines appear to converge to the values for the intrinsic constants. Fig. 7 demonstrates that until electrostatic effects are “peeled away”, titration curves for charged sorbents cannot yield intrinsic pK_a values. Therefore, electrostatic models are chosen in the attempt to account for the effects of the charged surface.

The “master curve” modelling approach developed by Kinniburgh, Koopal, Milne, Benedetti, van Riemsdijk, de Wit et al. and exemplified by de Wit et al. (1993a,b) is highlighted here to further the concepts illustrated in Fig. 7. This group applies a three-step approach. First, an appropriate electrostatic model is chosen, thus, establishing an expression for Ψ . Secondly, the original proton-binding curve data (net charge versus pH) is recalculated and sorbent charge is alternatively plotted versus pH_s , the negative log concentration of protons at the binding sites (Eq. (8)). This yields a single “master curve” (de Wit et al., 1990), where ionic strength effects are no longer evident. The master curve, however, still has a “smeared out” appearance that now reflects only the intrinsic heterogeneity of the sorbent. The heterogeneity of the sorbent is ac-

counted for in the final step, where analysis of the master curve results in a proton affinity curve that is represented by an appropriate site-binding model (continuous/discrete). This approach again demonstrates that models must, in essence, “peel away” the complexities associated with heterogeneous sorbents, beginning with the electrostatic effects and finally the intrinsic heterogeneity of the sorbent.

5.5.2. Simple ligand

At their most simple, models neglect electrostatic effects at the interface. Such models are empirical, and can be considered simply as curve-fitting equations (Perdue, 1985). The binding sites of the heterogeneous solid are assumed to be analogous to simple ligands in solution: thus, a discrete or continuous model is chosen to describe the experimental titration curve. Simple ligand models have been applied with success to proton and metal ion binding to bacterial surfaces (Cox et al., 1999), to NOM (Westall et al., 1995; Benedetti et al., 1995; Smith and Kramer, 1999a,b; Takács et al., 1999) and metal oxide surfaces (Borkovec et al., 1998).

The simple ligand approach does have utility under certain conditions. Perdue (1985) was not able to distinguish polyprotic acids from a mixture of monoprotic acids using only proton binding data. These results indicate that although there exists more than one functional group in polyelectrolytes such as humic acids, these groups are independent of each other and proton binding to one group is not affected by proton binding to another. In such cases, treating the polyelectrolyte simply as the sum of independent ligands is justified. Simple ligand models are also applicable under conditions of fixed pH and low metal ion sorption density (not approaching binding site saturation; Morel et al., 1981). Under these conditions, the charge on the sorbent is fixed by the extent of protonation/deprotonation of the surface functional groups and sorption of a small amount of metal ions has little effect on the sorbent charge. The now constant coulombic term (Eq. (6)), therefore, can be lumped with the intrinsic constant to yield an apparent, or conditional, equilibrium constant. In this case, the titration profile behaves like that of a simple ligand where each value of pK is shifted by a constant value representing the fixed potential at the

surface. Bourg and Mouvet (1984) and more recently Westall et al. (1995) have successfully used this approach to model metal ion binding to NOM. Similarly, correction for ionic strength effects involves a constant term to account for the screening of charge (discussed below).

5.5.3. Pure surfaces

Sorbents modelled as pure surfaces have been represented as impenetrable and infinite planes or as impenetrable spheres. EDL models are used to account for electrostatic effects on complexation reactions at these surfaces. All EDL models involve solving the Poisson–Boltzmann equation, which assumes that electrostatic potential arises from a centrally charged region about which, small, mobile ions in the solution arrange themselves in relation to the magnitude of the potential. The equation is composed of two parts, the Poisson equation relating potential to charge density and the Boltzmann distribution of co- and counter-ions in the potential field (Bartschat et al., 1992). Solving both parts of the equation permits description of the spatial distribution of co- and counter-ions in the interfacial region, and yields an expression for the surface potential as a function of distance from the charged surface.

5.5.3.1. Infinite plane—surface complexation models. The base model for metal oxide surfaces is the SCM. The historical development of the surface complexation model for iron oxide surfaces is a discussion beyond the scope of this review and can be found in Dzombak and Morel (1990). Important for our discussion here, is that this model treats the surface of metal oxides as infinite planes and that only one surface site type, described as hydroxyl groups, is considered necessary to explain their acid–base behaviour. The hydroxyl group is modelled as a diprotic acid; the neutral hydroxyl group, $\equiv\text{XOH}^0$, can be protonated, $\equiv\text{XOH}_2^+$, or deprotonated, $\equiv\text{XO}^-$, (where $\equiv\text{X}$ designates a surface species) as shown in Fig. 1. Iron oxide surfaces are thus characterized by two discrete pK_a values. The “best estimate” pK_a values for the protonation and deprotonation reactions at the surface of hydrous ferric oxide are 7.29 and 8.93, respectively (Dzombak and Morel, 1990). Metal ions are also deemed to

interact with the hydroxyl functional groups, as either inner or outer-sphere complexes. The relative positioning of the metal ion differs for inner and outer-sphere complexes due to the nature of the interaction; metal ions bound as inner sphere complexes are placed close to the surface sites while metal ions bound as outer-sphere complexes are placed some specified distance from the surface sites. Because surface complexation reactions result in a change in surface charge, and associated surface potential, EDL models for metal oxide surfaces must consider (1) protonated and hydrolysed surface species, (2) surface sites coordinated with major ions of the background electrolyte, and (3) surface-sorbate complexes (Morel et al., 1981). A discussion of specific EDL models follows.

For infinite planes, the Poisson–Boltzmann equation has an analytical solution derived by Gouy and Chapman (Morel and Hering, 1993). Surface charge is expressed as a function of surface potential (Eq. (9)).

$$\sigma_p = (8\varepsilon\varepsilon_0 RTI)^2 \sinh(\Psi F/2RT), \quad (9)$$

where σ_p is the net total particle charge, ε is the dielectric constant of water, ε_0 is the permeability of

free space and I is the ionic strength. Due to thermal motion, counter-ions do not form a plane of counter-charge (Stumm and Morgan, 1996), rather, the distribution of counter-ions, decays exponentially with distance from the charged surface (Fig. 8), and is characterized by the “double layer thickness” or Debye length, $1/\kappa$ (nm). For small potentials, the thickness of the double layer is linearly related to the ionic strength by

$$1/\kappa = 0.30 I^{-1/2}. \quad (10)$$

The double layer thickness expresses the distance from the surface over which counter-ions are accumulated and co-ions repelled by the charged surface (Morel and Hering, 1993).

The capacitance ($F m^{-2}$) is expressed

$$C = \sigma_p/\Psi = 2.3 I^{1/2}. \quad (11)$$

Referring to Eqs. (9)–(11), consider the acid–base titration of a metal oxide surface at different ionic strengths (Fig. 1a). The double layer thickness, surface charge, and surface potential are all related to the ionic strength of the solution. As the ionic strength increases, the EDL thickness decreases, and the sur-

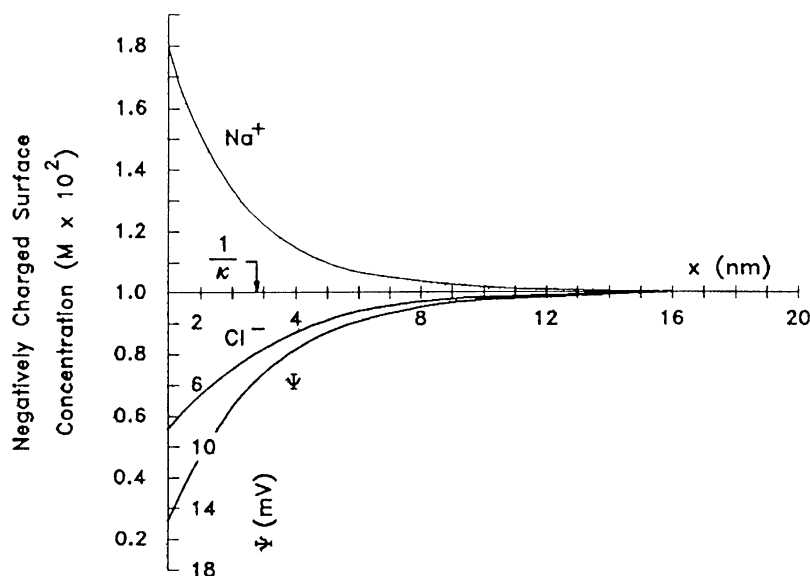


Fig. 8. Theoretical distributions of ion concentrations and electrical potential near a charged surface. (Calculated for the small potential approximation.) $1/\kappa$ is the thickness of the double layer; Morel and Hering, 1993, with permission*.

face charge (considering H^+ to be the only potential-determining ion) increases due to counter-ion shielding (e.g., at low pH, the electrolyte ions shield the positive charge of the surface resulting in less surface-proton repulsion and a greater protonation of the surface; Dzombak and Morel, 1990). At high ionic strength (≥ 1.0 M), the double layer is effectively so compressed that the interfacial region is “collapsed”. Because there is no longer a differential between sorbate concentration at the surface and in bulk solution, the interfacial potential becomes so infinitesimally small that its value approaches zero. This effect of high ionic strength applies not only to EDL models of pure surfaces, but to all electrostatic models of the interfacial region.

Double layer models. Models of the EDL at charged surfaces either ignore or variously differentiate between the location of potential-determining ions and counter-ions (for reviews of the individual models, see Westall and Hohl, 1980; Robertson and Leckie, 1998; Venema et al., 1996; Hayes and Leckie, 1987; Davis and Kent, 1990). However, all models

represent these areas by layers between the solid surface and bulk solution. The double layer model (DLM), also referred to as the diffuse double layer model (see Fig. 9a), represents the interfacial region with an inner layer comprising the potential determining ions and a second outer layer of counter-ions. The simplest model is the constant capacitance model (CCM; Fig. 9b). This model assumes that the interfacial region can be described by a single constant capacitance, i.e., is characterized by a linear relationship between surface charge and potential (Eq. (11)). In this case, all potential-determining ions are positioned in a plane directly parallel to the surface. Ions that are not adsorbed to the surface are not considered and therefore the surface potential is not a function of ionic strength. As such, the CCM has limited utility; it is applicable only under given chemical conditions. The triple layer model (TLM) represents the interface as a series of three layers (Westall and Hohl, 1980). The two layers closest to the surface are constant capacitance layers, and the outermost layer, the so-called diffuse layer. Different

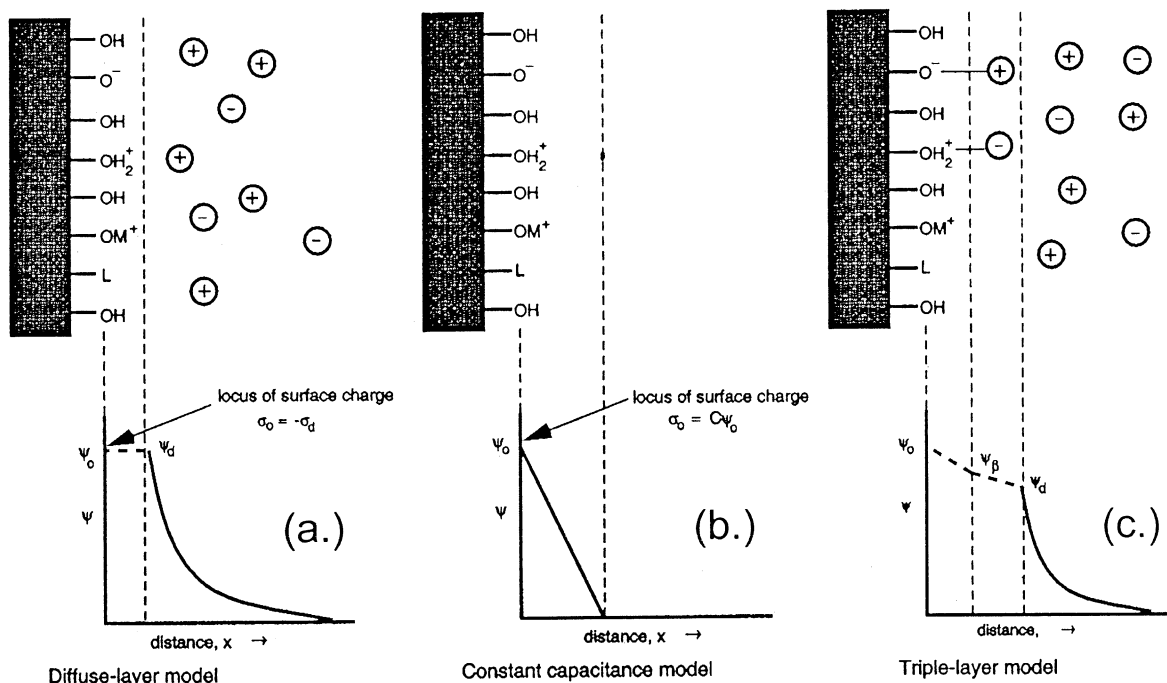


Fig. 9. Idealized distribution of electrical potential (Ψ) in the vicinity of a hydrated oxide surface according to (a) the diffuse-layer model, (b) the constant capacitance model and (c) the triple layer model (adapted from Drever 1997, with permission*).

researchers assign differently sorbed species to each of these layers, i.e., some assign inner sphere complexes, H^+ and OH^- ions to the innermost layer, where the ions experience the same potential, Ψ_0 (as shown in Fig. 9c). Species bound as outer-sphere complexes are positioned in the second layer and experience a potential of Ψ_β ; and co- and counter-ions are distributed in the diffuse layer according to the potential Ψ_d (Drever, 1997). Other researchers may restrict the innermost layer to H^+ and OH^- ions only, alternatively placing inner-sphere complexes in the second layer and outer-sphere complexes in the diffuse layer (Drever, 1997). These models are all referred to as DLM; although not all of them use a double layer to represent the solid–liquid interface.

The number of parameters to be optimized for each of the EDL models is different, and increases with the level of complexity of the representation of the interfacial region, i.e., the TLM involves optimization of a large number of parameters compared to the simpler CCM. Westall and Hohl (1980) state that all EDL models (they tested five) fit the experimental data and that one model was as good as the next, strictly in terms of “goodness of fit” to the data. The question then is whether the researcher is satisfied with strictly fitting the experimental data or, rather, is interested in gaining insight into the physical nature of the interface. This choice is discussed further (Section 5.7.1). It must also be considered that any model assuming a planar surface may be an oversimplification for the representation of metal oxide surfaces in nature, where any defect or three-dimensional aspects of a solid surface modify the local EDL (Parks, 1990; Banfield and Hamers, 1997).

5.5.3.2. Impenetrable sphere. Several groups have represented organic sorbents (mainly humic acids) as impenetrable spheres (de Wit et al., 1993a; Barak and Chen, 1992; Bartschat et al., 1992). The use of an impenetrable sphere as the basis of the electrostatic model for low molecular weight NOM (e.g., fulvic acids) is justified in a series of papers by Ephraim and Marinsky (1986), Ephraim et al. (1986), Marinsky and Ephraim (1986) and others (Bartschat et al., 1992; Kinniburgh et al., 1998). Using such a model, however, neglects the potential effects of polydispersity and conformational change of the molecule with changing chemical conditions (Milne

et al., 1995b), which may result in differing reactivity of the sorbent.

The non-linear forms of the Poisson Boltzmann equation do not have analytical solutions, and therefore must be optimized numerically. The numerical solution for a sphere expresses Ψ as a function of r (the distance from the center of the sphere) for a given ionic strength, molecular radius and surface charge density of the sphere (Bartschat et al., 1992). Therefore, molecules that have a larger radius have a greater (more negative) surface potential and related surface charge density. This is due to the convenient forcing of the total molecular charge, in reality spread over the whole volume of the sorbent, onto the surface of the spherical molecule. Since the volume of a sphere increases faster than its surface area by a factor of r , it follows that a sorbent with greater volume would have a greater surface charge density. The result, therefore, that larger (higher molecular weight) NOM molecules have greater surface potentials has implications for metal binding to NOM (Section 5.6.3).

An advantage to treating NOM molecule as an impenetrable sphere is that the average particle radius is the only dimension that needs to be set in this model to calculate the uniform electric potential (Kinniburgh et al., 1996; Milne et al., 1995b). The impenetrable sphere model is however disadvantaged by the need to solve the non-linear Poisson–Boltzmann equation numerically. This solution is computationally intensive, especially when a mixed salt solution is considered, and could therefore make this model unsuitable for chemical transport models (Kinniburgh et al., 1998).

5.5.4. Polyelectrolyte: Donnan-type models

Polyelectrolyte models employ the concept of Donnan equilibrium. A Donnan equilibrium can be explained most simply by consideration of a semi-permeable membrane that has electrolyte solutions of different chemical compositions but the same ionic strength, Na^+Cl^- and Na^+X^- on either side. If the membrane is impermeable to X^- , diffusion of Cl^- across the membrane will result in an equal diffusion of Na^+ across the membrane in the same direction, to maintain electrical neutrality. The final concentration of Cl^- , however, will be lower on the side of the membrane containing X^- due to the presence of

the fixed negative charge. The concentration difference created between the two sides of the membrane gives rise to an osmotic pressure and an electrical potential difference, Ψ (Everett, 1988). In the case of polyelectrolytes, a gel of polymer chains containing fixed binding sites creates a charged domain. A Donnan potential arises when a strong ion penetrates into this domain.

Donnan-type equilibrium models are well suited for the description of both high molecular weight NOM polyelectrolytes, such as humic acids, and bacteria. Humic acids, linear polyelectrolyte coils, are large enough to spread over a considerable volume and may encompass a significant amount of solvent. As such, a boundary, analogous to the semi-permeable membrane discussed above, can be imagined that surrounds the molecule in three-dimensional space, creating a volume around the

molecule, the Donnan volume (Fig. 10). Analogously, bacterial cell walls have molecular-sieving properties, i.e., they are penetrable. Counter-ions may penetrate the cell wall matrix, with simultaneous expulsion of co-ions, and the volume between the cytoplasmic membrane, and the cell wall reaches Donnan equilibrium with the external environment (Ferris and Beveridge, 1985; van der Wal et al., 1997).

The Donnan volume is affected by pH and ionic strength-dependent conformation changes, i.e., physical extension, of the NOM polyelectrolyte in solution (as discussed in Section 4.2.6). Benedetti et al. (1996) reported that a linear relationship exists between the logarithms of the Donnan volume and solution ionic strength for humic and fulvic acids. The Donnan volume was also shown to be a function of ionic strength for bacteria (Plette et al., 1995). For

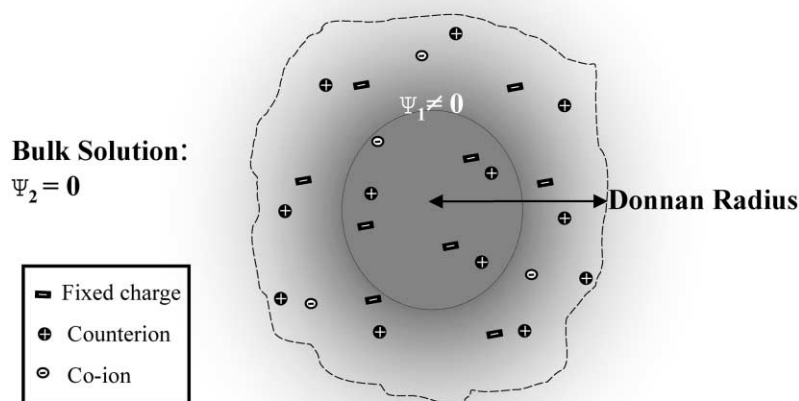


Fig. 10. The Donnan model conceptualization of an NOM molecule in bulk solution. The NOM molecule itself is here represented as a sphere situated inside a larger volume element (i.e., interfacial region), as indicated by the Donnan radius. The NOM molecule, which is the inner sphere in the figure, is represented here as such only for the sake of simplicity. NOM molecular conformation is both pH and ionic strength dependent; and thus, the physical reality of such solids can take a wide variety of shapes (including diffuse agglomerations of several NOM molecules). The fixed charge associated with the penetrable solid is strictly positioned only within the limits of the sorbent (i.e., the penetrable NOM molecule shown here as a sphere). However, for modelling convenience, the Donnan approach assumes a uniform fixed charge throughout the whole Donnan volume. Both the co- and counter-ions can diffuse into the Donnan volume from bulk solution, and diffusion of negative charge across this “semi-permeable” membrane causes a concentration difference to be established between the Donnan volume and the bulk solution (see text for further explanation), which results in a non-zero potential ($\psi_1 \neq 0$) within the Donnan volume (where the bulk solution is defined as having $\psi_2 = 0$). The potential, ψ_1 , is modelled as being uniform over the whole Donnan volume.

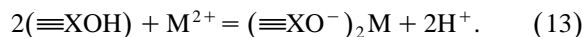
simplicity, a uniform distribution of binding sites is assumed over the whole Donnan volume, resulting in a uniform spatial charge density and a constant Donnan potential throughout the volume (Plette et al., 1995). Because of this uniform potential, the concentration of counter-ion species in the Donnan volume is easily accessible as its concentration in the bulk solution multiplied by the Boltzmann factor (analogous to Eq. (7)). In this manner, ionic strength, Donnan volume and Donnan potential are all related. The Donnan volume decreases with increasing ionic strength, resulting in a greater Boltzmann factor and a greater absolute value of the Donnan potential (Kinniburgh et al., 1999), until collapse of the volume occurs at high ionic strength.

There are several advantages to the Donnan model from a modelling perspective. First, the Donnan model does not assume a particular geometrical conformation for humic particles (Kinniburgh et al., 1999), which are highly variable in nature. This is in contrast to the diffuse double layer, in which the potential varies as a function of distance from the surface of the particle, and therefore is dependent on the specific geometry of the particle. Secondly, the Donnan model is easily added to a macro-model as it is not parameter intensive and the amount of each metal ion bound as counter-ions is very easily calculated (Kinniburgh et al., 1999). Schiewer and Volesky (1997a,b) successfully modelled both proton and cation binding to *Sargassum* seaweed mass and alginate using one fitting parameter for the Donnan model. Thirdly, the Donnan effect can be suppressed if the ionic strength is sufficiently high, as discussed above, and earlier for pure surfaces. Many studies have used proton-binding curves at high ionic strength (at least 1.0 M) to represent proton binding without electrostatic effects and have derived Donnan potential correction terms from such curves for binding at lower ionic strength (Plette et al., 1995; Marinsky et al., 1999).

5.6. Sorbate: metal ions

In extending models from the description of proton binding to the description of metal ion sorption, two considerations must be addressed. The first is whether or not the binding of the metal occurs at the same site as the protons, i.e., is the binding competi-

tive? The second consideration is the stoichiometry of the metal binding. Monodentate (1:1) and bidentate (1:2) binding of a divalent metal ions are expressed, respectively, as



Both monodentate and bidentate bindings are illustrated in Fig. 1. In the strictest sense, not only monodentate and bidentate bindings of metal ions should be considered in geochemical models; but also multidentate binding of the metal ions, as well as binding of hydrolysed species (e.g., CdOH^+). If monodentate metal ion binding occurs competitively at proton-binding sites, then the maximum number of binding sites for that metal ion corresponds to the maximum number of proton binding sites (assuming all protons can be displaced; Kinniburgh et al., 1999). The total number of metal ions that can be bound decreases if the binding is bi- or multi-dentate as multiple sites are needed to bind a single metal ion.

The molar exchange ratio, H^+/M^{z+} , expresses the number of protons released from the sorbent as a function of the number of metal ions bound. Non-stoichiometric exchange ratios have been observed experimentally for NOM and oxide surfaces (Dzombak and Morel, 1990; Kinniburgh et al., 1998). For humic acids, exchange ratios over a pH range of 4–8 increased from approximately 1.3 to 1.7 for Cu^{2+} (Benedetti et al., 1995; Tipping et al., 1995; Robertson, 1996) and from 0.25 to 0.4 for Ca^{2+} between pH 6 and 8 (Benedetti et al., 1995). Exchange ratios for metal ions with metal oxide surfaces are generally higher than for NOM (Kinniburgh et al., 1983, 1998). Reported exchange ratios for most divalent cations with metal oxide surfaces, vary somewhere between 1.6 and 2.0 at all pH and ionic strengths (Benjamin and Leckie, 1981; Dzombak and Morel, 1990; Robertson and Leckie, 1998). Alkaline earth metals give lower exchange ratios; an exchange ratio of ~ 0.9 was found by Kinniburgh et al. (1983).

Thermodynamic analysis of the molar exchange ratio relationship is based on what is called thermodynamic consistency (Černík et al., 1996; Borkovek et al., 1998). In essence, thermodynamic consistency states that if the activity of one surface species is

varied in solution, keeping everything else constant, the resultant change in surface charge will always be the same, regardless of which species activity is varied. Therefore, as the bulk activity of one species is increased, a corresponding loading of that species at the interface is expected. What this means for models of competitive metal binding is that only models that are thermodynamically consistent are able to equate experimentally measured exchange ratios in the bulk solution to exchange ratios at the interface. For example, if an exchange ratio for Cu^{2+} was measured as 1.7 with HA, only a thermodynamically consistent model could confidently relate that ratio to the actual release of 1.7 protons from the surface for every metal ion bound. Therefore, a thermodynamically consistent model is one in which the pH dependence of the metal ion binding is directly related to the number of protons released per metal ion bound (Kinniburgh et al., 1999). The exchange ratio is important when predicting the change in system pH with high metal loadings (Kinniburgh et al., 1999).

5.6.1. Monodentate binding

There are several ways to account for the competitive binding of metal ions to sorbents. The simplest manner is to assume that the metal ion binding is monodentate (Eq. (12)), i.e., analogous to proton binding, and that the metal ion affinity distribution is equivalent to the proton affinity distribution, but shifted along the pK axis (Milne et al., 1995a). Such models for organic sorbents (Dobbs et al., 1989; Manunza et al., 1995) are said to be fully correlated or congruent (Kinniburgh et al., 1998). Correlated distributions have been shown to satisfactorily describe the binding of some metals, e.g., Ca^{2+} and Cd^{2+} to peat purified humic acid, however, fail to describe the high exchange ratio and strong pH dependence of Cu^{2+} sorption to the same humic acid (Milne et al., 1995a). In effect, fully correlated models suppress any heterogeneity effects of metal ion binding to a sorbent, i.e., they do not take into account that the exchange rate of protons per metal sorbed is metal specific and not equivalent for all metals.

5.6.2. Multidentate binding

Another approach taken to modelling metal sorption to organic surfaces allows sorption to be either

monodentate (1:1) or bidentate (2:1). Model V (Tipping and Hurley, 1992) is one such model. Model V can be considered intermediate between continuous and discrete models; the number of sites is fixed but a combination of two sites allows for bidentate metal binding. An empirical term was incorporated into this model to account for electrostatic effects and a Donnan-type expression accounts for the accumulation of counter-ions. The advantage to Model V is that while it incorporates both electrostatics and heterogeneity effects, it does not require elaborate numerical solution techniques, e.g., it is easy to use and robust in its estimations (Santschi et al., 1997). Further, it has been incorporated into Windermere Humic Acid Model (WHAM; Tipping, 1994) and the Surface Chemical Assemblage Model for Particles (SCAMP; Lofts and Tipping, 1998). SCAMP is a macro-model comprising both SCM (oxides) and Model V (organics) subunits to allow for differences in sorbent reactivities. The advantage to a macro-model like SCAMP is clear; it allows for simultaneous modelling of metals to mixed mineral organic solids. This model has given good fit to the sorption of nine metals exhibiting very different affinities for oxides and organics as well as potentially different stoichiometries of association (Co, Ni, Cu, Pb, Zn, Sr, Cd, Cs, Eu) to both riverine and estuarine heterogeneous suspended particulate matter; emphasizing the strength of this approach.

The last class of models takes the most general approach in which no a priori assumptions concerning the stoichiometry of the metal surface functional group complex is made. The most deterministic of semi-empirical models that accounts explicitly for competitive metal binding is the NICCA–Donnan model. The NICCA–Donnan model, developed by Kinniburgh et al. (1999) is an extension of the earlier continuous NICA (non-ideal competitive adsorption) model (Koopal et al., 1994). The NICA model recognizes that the affinity distributions are metal ion specific and not fully correlated with either protons or other metals (Kinniburgh et al., 1999). The weakness of the NICA (no electrostatic correction) and later NICA–Donnan models (Kinniburgh et al., 1996) is that they are not thermodynamically consistent. The NICCA (the consistent NICA)–Donnan model accounts for both electrostatic effects (Donnan model) and the intrinsic binding heterogeneity of

humic materials. Several aspects distinguish the NICCA–Donnan model from surface complexation models and other models such as Tipping and Hurley's Model V: (1) the NICCA–Donnan model is thermodynamically consistent (hence, the name); (2) it does not make any a priori assumptions about the binding stoichiometry; (3) it assumes a partial correlation between the proton and metal affinity distributions; and (4) most importantly, the model-derived site densities and median binding constants are not dependent on any solution conditions (e.g., pH, ionic strength, metal ion concentration, etc.). This last characteristic means that binding can be predicted without knowing system conditions other than the sorbent and the specific metals of interest. Further, using only parameters derived from the pure $[M^{2+}]$ (Ca, Cu, Cd, Al, Pb) sorption data, the model was able to make reasonable predictions for the competitive binding between Ca and Cu, Ca and Cd, and Al and Pb to HA (Kinniburgh et al., 1999). The strength of this approach therefore is twofold; simplicity and ease of use, and predictive ability. A model such as this, which can be calibrated with single metal ion systems but can predict the sorption behaviour in mixed systems, is extremely useful.

5.6.3. Model implications

Two important trends that have implications for metal sorption to sorbents in natural aquatic environments have emerged from competitive metal-ion models. The first applies to all sorbents; the types of binding sites needed to model metal binding compared to proton binding have to be considered. Secondly, the size distribution of NOM particles also has to be accounted for as it relates directly to NOM proton and metal-binding affinities.

In general, with continuous models for NOM, proton-binding data clearly show that the proton affinity distribution is essentially bimodal (reflecting distinct binding sites thought to represent carboxyl and hydroxyl groups) and that two broad distributions are required to describe these data accurately (Kinniburgh et al., 1999). However, when modelling metal sorption to organic surfaces, it often appears that carboxylic-type sites absolutely need to be considered and that depending on the sorbent, e.g., bacteria or NOM, other binding sites may also have to be considered. Only carboxylic-type sites were

reported to be necessary to give a good fit to the experimental data for Ca^{2+} and Cd^{2+} to humic acid, whereas both carboxyl and phenolic-type sites were needed to reproduce the acid–base behaviour of the same sorbent (Milne et al., 1995a). Westall et al. (1995) demonstrated that only two sites (pK_a s 6 and 8) were needed to account for the large effect of ionic strength on the Co–HA interactions. Marinsky et al. (1999) assumed calcium ion binding to the fulvic acid occurred only at carboxylate-type groups and fixed four pK_a values. In a suite of papers describing metal sorption by bacteria, Fein et al. (1997), Daughney and Fein (1998b) and Daughney et al. (1998) found that three binding sites were needed for proton binding to two Gram-negative bacteria, inferred to be carboxyl, phosphate and hydroxyl functional groups. Yet, only two sites were needed for metal binding, inferred to be carboxyl and phosphate groups. These findings highlight the important and possibly predominant role that carboxylic functional groups play in controlling sorption of metals by organic surfaces. This has to do with both the pK_a values of carboxylic groups, typically in the range of 4–6, and the pH range of natural aquatic systems. Other potential binding sites such as phosphoryls ($pK_a \sim 7$) and amines or phenols (> 9) are often not substantially deprotonated in natural environments.

For metal oxide surfaces, a good description of metal binding generally requires two binding sites of different, high and low, affinities. Dzombak and Morel (1990) found that the position of the adsorption edge (% ion sorbed versus pH) shifted to higher pH values as the metal ion/sorbent ratio was increased, meaning that at a given pH the fraction of metal ion bound is decreased. This trend indicates that metal ion sorption density is not proportional to dissolved metal ion concentration (except at very low sorbate/sorbent ratios); i.e., that the sorption affinity for the metal ion decreases as the dissolved metal ion concentration is increased. The authors state that this trend can follow from either a multiplicity of sites or saturation of available sites. They reported an approximately equal site saturation for metal ion sorption as for proton binding. This indicates that the total concentration of metal ion-binding sites is the same as the total concentration of proton-binding sites. Therefore, two monodentate

sites were created to describe the metal ion sorption, one of high affinity and one of low affinity. The proportional sorption, at low sorbate/sorbent ratio, is ascribed to metal ion binding to the high affinity sites. Once these sites are saturated, the metal ion sorption is non-proportional and is ascribed to the binding of the metal ions to the low affinity sites. Robertson and Leckie (1998) also needed to incorporate two binding sites of different affinities in their surface complexation model. The need to represent more than one binding site on metal oxide surfaces may seem intuitive, considering that natural oxides: contain extensive structural and surface impurities; are polycrystalline; are typically aggregated; and exhibit a range of solubilities, defects, and coatings (Brown et al., 1999a,b). However, creation of such sites to account for the features of the adsorption isotherms, again, requires justification from spectroscopic evidence or known properties of the solid.

Size heterogeneity is another important consideration for both proton and metal binding to NOM, as it will reflect not only the types and densities of associated functional groups, but also the size-related effect of ionic strength. The ionic strength effect results from employing a spherical geometry to describe the NOM particle, and as such, forcing the molecular charge to reside at the surface of an impenetrable sphere, as discussed earlier. de Wit et al. (1993a) found that ionic strength effects on proton sorption to humics increased with increasing molecular weight. Bartschat et al. (1992) reported a large ionic strength effect for Cu^{2+} sorption to FA, but only a relatively small ionic strength effect on proton sorption. These ionic strength effects were ascribed to the preferential binding of protons and metal ions to different size fractions of the FA sample. Larger spherical surfaces have a more negative surface potential and therefore the higher molecular weight fraction binds Cu^{2+} preferentially. In contrast, protons were preferentially bound by the smaller and more abundant weight fraction. Therefore, the effect of NOM particle size not only influences sorption behaviour of metals but also the attachment of NOM to other solids. Larger NOM particles are preferentially sorbed to mineral surfaces and themselves preferentially sorb metal ions. Thus indicating that, the larger the NOM size the more likely that metals will partition to the solid phase.

5.7. Comparison of models

All the models give satisfactory “fit” to the experimental data under a given set of conditions, in many cases using correction terms to move from a more parameter intensive and in some cases more deterministic model, to a less parameter intensive, and more empirical model. The relative validity of each of the models, therefore, must certainly be considered in terms of the application. Implicit in the selection of a model is an understanding of the assumptions of that particular model, and therefore its suitability for a given set of data. The critical considerations when selecting a model are: the number of components, e.g., one metal and three sorbents versus three metals and three sorbents; which metal(s) is/are of interest, and their binding stoichiometry; system pH and ionic strength conditions, and how variable these parameters are. As stated by Kinniburgh et al. (1999), a fully mechanistic approach to modelling heterogeneous sorbents, without making arbitrary assumption, remains “Utopian for the foreseeable future”.

5.7.1. Intrinsic limitations of potentiometric titrations

A fundamental limitation of all the modelling techniques discussed here is that they are based on potentiometric titrations. Smith et al. (1999) demonstrated that the greatest uncertainty in parameter estimations from acid to base titrations occurred outside of the pH range 4–10 for an FA sample. Although there may be binding sites outside of this range, they are not accessible with glass electrodes. The authors state that it would be necessary to increase the sorbent concentration above levels found in natural waters to reduce these uncertainties. Because of such pH range limitations, potentially very high affinity (high $\text{p}K_a$) and very low affinity (low $\text{p}K_a$ values) are not accessible (Martinez et al., 1998; Kinniburgh et al., 1999), and it is not possible to observe cation binding to uncharged NOM.

Perdue (1985) states that progressively more complex mixtures demonstrate titration curves that become increasingly “smeared out” or featureless. The ease of fitting a mathematical model to the smoother curve, or “curve-fitting”, also increases. Therefore, it

becomes more difficult to use “goodness of fit” to determine whether the mathematical model, based on a few adjustable parameters, is also a sound chemical model.

Moreover, it may be that we are not able to differentiate between models for the EDL with potentiometric techniques, i.e., we may not be able to make any conclusion about the electrostatic interactions at the solid–liquid interface beyond what can be obtained by a good fit. Westall and Hohl (1980) determined that models of the EDL employed in surface complexation models could be used to fit the experimental data but that none of them yield an unambiguous description of the adsorption energies at the solid–liquid interface. The fundamental properties are, however, real since recent atomic force microscopy studies of the EDL have confirmed an exponential dependence of the forces acting on the tip as a function of the distance of the tip from the metal oxide surface, and that the double layer thickness does decrease with increasing ionic strength (Brown et al., 1999a,b). Further spectroscopic investigations will permit us to better probe the microscale interactions of the solid–liquid interface (see Section 7.4), and potentially determine the suitability of our model hypotheses.

5.7.2. Continuous versus discrete models

Continuous models give a more intuitive description of metal and proton binding to heterogeneous sorbents such as humic materials, as it is easy to view such complex macromolecules as having an infinite range of surface functional groups due to their polyfunctional properties (involving the chemical nature of the site, and the electronic and steric environment), polyelectrolyte properties and changes in conformation (Buffle, 1990). Residuals analysis has shown that the continuous models are probably closer to reality than discrete models, even when quite a large number of sites are used (Kinniburgh et al., 1999). In general, discrete models are considered advantageous as only discrete sites can be incorporated easily into geochemical code calculations (Černík et al., 1995; Cox et al., 1999). However, these models may be less accurate because of the use of a discrete (fixed) number of sites. For instance, Martinez et al. (1998) compared continuous and discrete models for the description of potentiometric

titrations of polyacrylic acid (PAA—a HA analogue). A three binding site discrete model was required to give a good fit to the experimental acid–base titration of PAA. PAA is a monofunctional (carboxylic) synthetic polymer (Martinez et al., 1998), and therefore multiple binding sites for this monofunctional molecule seem unlikely. The least abundant of the binding sites had a pK_a value of 7.57 which, again, does not correspond to the carboxylic functional group making up PAA. Moreover, pH-dependent molecular conformational change, which could result in binding sites of different reactivities, is also not likely for this molecule. In contrast, a monomodal continuous model gave an adequate fit to the experimental data and the distribution of pK_a values was within the expected range for the carboxyl functional group of PAA. This study suggests that discrete models do not accurately reflect the physical nature of the sorbent, even if the model fit is good. As such, discrete models should be incorporated into larger metal speciation framework with some caution.

5.7.3. Impenetrable surfaces versus penetrable gel-phases

With regards to modelling surfaces, the SCM is well established and has been used extensively. SCM models have successfully described proton and metal ion binding to bacterial surfaces, to humic substances (Ganguly et al., 1999), to kaolinite (Schindler et al., 1987; Payne et al., 1998), to goethite (Robertson and Leckie, 1998), to natural sediment (Wang and Chen, 1997; Wen and Tang, 1998), to hematite in the presence of humic acid (Au et al., 1999; Lenhart and Honeyman, 1999), and to riverine and estuarine SPM (Lofts and Tipping, 1998), and this list is certainly not exhaustive. However, although this model is well established, Marinsky (1996) states that the two-dimensional triple layer SCM provides an incorrect assessment of the function of the counter-ion with regards to ionic strengths effects on proton binding curves. He proposes that hydroxylated metal oxide particles, which in nature have heterogeneous geometry and are porous, should alternatively be modelled in three-dimensions. Using the three-dimensional approach, the particle is assigned separate phases, equivalent to the Donnan model for NOM particles. Krapiel et al. (1998) also state the traditional SCM models do not give good results for geochemically

reactive solids such as clays that possess a permanent pH-dependent charge. They suggest that the model for such minerals should allow for penetration of the solid surface by electrolyte ions, resulting in what can be termed a “double–double layer”. In effect, a Donnan potential is established inside the porous particle (at large potentials) and a diffuse layer is established at the solid surface to describe the distribution of ions between the interface and the bulk solution. Using this model, the important features of the acid–base titration of Na-montmorillonite were well described. Therefore, the Donnan model is applicable to reactive solids beyond high-molecular weight NOM, and as stated earlier, this model is not parameter intensive.

5.7.4. Models for proton and metal sorption to bacteria

5.7.4.1. Proton binding. As bacteria are the focus of the remainder of this review, the implications of the different models for bacterial surfaces are described in more detail here below. All three mechanisms have been applied to modelling proton (and metal ion) binding to bacteria: bacteria have been modelled as pure surfaces (SCM), as penetrable polyelectrolytes (with the Donnan model), and as simple ligands.

Table 1 gives proton-binding constants and site densities for three bacterial species taken from the literature. The acidity constants for derived for *B.*

Table 1
Summary of pK_a values and site densities determined by three different modelling approaches for three strains of Gram-positive bacteria

	Plette et al., (1995) (Master curve approach)	Cox et al., (1999): discrete affinity spectra	Fein et al. (1997): SCM	Daughney et al., (1998): SCM
Electrostatic model	Donnan equilibrium	None	Constant capacitance (CC)	CC
Equilibrium constants	Intrinsic	Apparent	Intrinsic	Intrinsic
Model type	Continuous	Discrete	Discrete	Discrete
Bacterial species	<i>R. erythropolis</i> (cell wall)	<i>B. subtilis</i> (whole cell)	<i>B. subtilis</i> (whole cell)	<i>B. licheniformis</i> (whole cell)
Ionic strength	Effects corrected	0.1 M, 0.025 M	Effects corrected	Effects corrected
Site	pK_a site density ($\mu\text{mol}/\text{mg}$)			
1	4.62 ± 0.02 , 0.53 ± 0.015	5.25 ± 0.03 , 5.17 ± 0.01 , 0.16 ± 0.02 , 0.17 ± 0.04	4.8 ± 0.1 , 0.12 ± 0.01	5.15 ± 0.3 , 0.088 ± 0.038
2		6.00 ± 0.00 , 5.88 ± 0.01 , 0.01 ± 0.02 , 0.05 ± 0.05		
3	7.83 ± 0.13 , 0.193	6.69 ± 0.01 , 6.91 ± 0.05 , 0.01 ± 0.01 , 0.05 ± 0.02	6.9 ± 0.3 , 0.04 ± 0.02	7.47 ± 0.4 , 0.0834 ± 0.046
4		8.33 ± 0.01 , 7.88 ± 0.04 , 0.07 ± 0.01 , 0.01 ± 0.01		
5	9.96 ± 0.08 , 0.235 ± 0.007	9.95 ± 0.06 , 9.24 ± 0.07 , 0.25 ± 0.03 , 0.12 ± 0.01	9.4 ± 0.3 , 0.062 ± 0.02	10.17 ± 0.05 , 0.127 ± 0.068
Total site density	0.96	0.5, 0.4	0.2	0.3

subtilis by the SCM model and the discrete affinity spectra model are within 0.5 log units of each other. It must be noted that the differences in these values are due to the modelling technique: the SCM model derives intrinsic constants (constants extrapolated to zero ionic strength), while the discrete affinity spectra model derives only apparent constants. All three bacterial strains show three common site types with pK_a values at ~ 5 , 7 and 10. These sites were inferred to represent carboxylic, phosphate and amine (Plette et al., 1995; Cox et al., 1999) or hydroxyl (Daughney et al., 1998) groups.

As shown in Table 1, Daughney et al. (1998) found differences in the site densities and pK_a s between *B. subtilis* and *B. licheniformis*. They suggest, however, that the chemical variations between them are of little statistical significance, and that it may therefore be possible to select a single, average set of surface proton binding sites to describe experimental acid–base behaviour of complex bacterial populations. That the acidity constants for the three common sites between the three models (Master curve, SCM and discrete affinity spectra) are comparable (within 1 log unit) further supports this suggestion. Daughney and Fein (1998b) further reported an excellent correlation between the metal-carboxyl and metal-phosphate stability constants between *B. subtilis* and *B. licheniformis* at given solution conditions. The authors suggest that as a result of this correlation, the metal-carboxyl stability constant for one species of bacteria could be estimated if the metal-carboxyl stability constant for another species (with the same metal) has been measured.

5.7.4.2. Metal binding. Fowle and Fein (1999a) demonstrated that hysteresis was not observed for the adsorption of Ca^{2+} and Cd^{2+} to bacteria and therefore the adsorption process is fully reversible. These results indicate that metals ions are bound at the surface of the cell walls and are not transported into or through the cell walls.

Plette et al. (1996) found that the exchange ratio for Cd^{2+} to the cell walls of *R. erythropolis* A177 was between 0.5 and 0.25 (for pH 5, 6 and 8), using the NICA model. Also reported was the increased binding of both Cd^{2+} and Zn^{2+} in the presence of Ca^{2+} . The authors were not able to ascribe this increase to a specific mechanism. They

did, however, suggest that the increased metal ion binding in the presence of Ca^{2+} occurred specifically at phosphoryl sites; due to either changes in cell wall structure or reactive group conformation associated with the sorption of Ca^{2+} to the cell wall. Plette et al. (1996) also reported that charge reversal (from negative to positive) took place for the bacterial cell in the presence of Ca^{2+} and high coverage of the surface with bivalent cations. This result has implications for the binding of bacteria to negatively charged mineral surfaces in natural environments, i.e., instead of being repelled by the surface; the positively charged bacteria would be electrostatically attracted to the mineral surface. Collins and Stotzky (1992) also observed charge reversal for five different bacteria at pH 6–10, but ascribed the reversal to the adsorption of hydrolysed species (e.g., $CuOH^+$). Fowle and Fein (1999b) demonstrated that the inclusion of carboxyl- and phosphoryl-metal hydroxide complexes into their SCM model for *B. subtilis* and *B. licheniformis* improved model fit to the experimental data at pH above 5. These results have important implications as the toxicity of metals depends on their speciation. The sorption of more than one species of a single metal to the bacterial cell wall may potentially place the organism at risk of exposure to a more toxic species.

5.8. Comparing laboratory- and field-derived equilibrium constants

Comparisons of laboratory and field derived equilibrium constants are important accuracy-determining components of geochemical models; but are rare in the literature. We could only find one paper that actually compared their field estimates to experimentally derived values. Tessier et al. (1996) compared the equilibrium constants for metal (Ca, Cd, Cu, Ni and Zn) sorption to Fe and Mn oxyhydroxides and NOM from two lakes, one acidic (4.8) and one circumneutral (7.4). Experimental metal–mineral (Fe and Mn oxyhydroxides)-binding constants were compared to those binding constants that could be predicted in the laboratory using the double layer SCM. For circumneutral lake, a significant correlation was established between the measured conditional constant and the predicted intrinsic equilibrium constant. This result was consistent with their

other experimental results, which indicated that in this system the metals were bound directly to the surface functional groups of the oxyhydroxide particles. For acidic lake, the measured conditional equilibrium constants had values greater than that predicted by the SCM-DLM. The Windermere humic acid (WHAM) equilibrium model (Tipping, 1994), was then used to calculate the conditional constants for metal binding to both dissolved natural organic matter and natural organic matter adsorbed to the Fe oxyhydroxides. The model-derived constant results showed similarity for the metals Cd, Cu, Ni and Zn. This result supported their hypothesis that the metals were bound to the humic substances associated with oxyhydroxide particles in the acidic system.

5.9. Conclusion

Geochemical models, whether deterministic or empirical, discrete or continuous, must account for both the inherent physico-chemical complexity of natural sorbents and the limited information content of measurable experimental data (Buffle, 1990). The importance of the model to accurately describe the acid–base behaviour and metal sorption to the solid is clear; our prediction of the fate of metals in natural and complex systems is only as good as the binding constants we have predicted, which are only as good as the models we have developed, which are in turn only as good as the experimental data (Martinez et al., 1998).

Oreskes et al. (1994) outline some general concerns on numerical geochemical models that seem fitting to our discussion. The authors state that the primary value of models is stated to be heuristic; i.e., that they are representations that focus future study and hypotheses but cannot be used as proof of any given process. Further, models must not be used to “confirm” results that may represent experimental (or personal) bias. Therefore, the utility of models lies only in the examination of the extent of a hypothesis, not in the establishment of the validity of a model based on “goodness of fit”.

Oreskes et al. (1994) also mention that a common assumption in many geochemical models of water–rock interactions is that observable mineral assemblages achieve equilibrium with a modelled fluid

phase (we have made this assumption in our discussion). To include kinetic factors would mean more assumptions and inferences as relevant kinetic data are frequently unavailable. Kinetic effects are most often assumed to be negligible. In our introduction to this discussion on modelling techniques, we stated that one of the goals of a modelling approach is to address the question “How does time affect the establishment of equilibrium partitioning of the sorbent between the solid and liquid phases?” Brassard et al. (1996) found that the exchange of protons with natural sediment particles requires equilibration times as long as 2–6 h. This study has implications for metal binding to heterogeneous, porous and particles.

Brown et al. (1999) comment that modelling techniques should be predictive over a wide range of solution environments, including variations in temperature. They were only able to site one study of the effects of elevated temperature on the protonation of rutile (Machesky et al., 1994). Warren and Zimmerman (1994b) identified temperature to be very significant in determining the extent of Cd, Cu and Zn partitioning between the total particulate pool and the dissolved phases in a field study of a contaminated river. In contrast to the results outlined above, Puranik and Paknikar (1999) found that metal uptake by *Citrobacter* strain MCM B-181 (Gram-positive) was rapid, requiring less than 30 min (30, 15 and 10 min for Pb, Cd and Zn, respectively), regardless of whether the bacteria was immobilized in pellet form.

In conclusion, models that assume equilibrium conditions are certainly simplified representations of natural systems and, as such, are operationally defined. It follows that the extension of models to natural systems necessarily requires more knowledge of the kinetics, and associated temperature effects, of interactions at the solid–water interface as suggested over a decade ago by Honeyman and Santschi (1988).

6. Microbial ecology: geochemical implications

Historically, our quantitative understanding of metal behaviour has developed from a strict geochemical perspective. However, the overwhelming importance of bacteria as a key player in determining both metal behaviour and impact, is becoming in-

creasingly evident. Bacteria actively influence the partitioning of metals between solid and dissolved phases through their control on mineral formation and dissolution reactions, as well as through their ability to change the chemical conditions of their environment through natural metabolic processes. The magnitude of such changes and thus impact on solid solution partitioning will be directly linked to the intensity and location of microbial activity. Bacteria are particularly important in influencing environmental geochemical processes because the smaller the size of a cell, the higher the metabolic activity (Holt and Leadbetter, 1992). For instance, the volume of a typical 1- μm prokaryotic cell is approximately 8000 times smaller than that of a typical 10- μm -sized eukaryotic cell; however, the surface area to volume ratio of the prokaryotic cell is about 20 times greater. Consequently, bacteria can consume nutrients and produce metabolic products at far greater rates than eukaryotic cells. Thus, their likely impact on processes is orders of magnitude greater.

Any bacterially promoted changes in either mineral formation or dissolution will likely impact subsequent metal reactions through associated changes in the relative amount of solid surface and associated functional groups available for metal complexation. Bacteria influence these processes through a variety of mechanisms, all related to bacterial metabolic processes. Further, bacteria influence these processes at both microscopic, e.g., weathering of mineral surfaces, and macroscopic, e.g., geochemistry of the system, scales (Bennett et al., 2000).

6.1. Biomineralization: microbially promoted mineral formation

Biomineralization can occur actively, i.e., “biologically controlled”, and passively, i.e., “biologically induced” (Lowenstam, 1981). Bacterial cells that control mineralization actively regulate the precipitation of physiologically essential minerals. In this way, minerals that are not thermodynamically favorable are formed by introducing specific ions either intracellularly or extracellularly in a developed organic framework (e.g., magnetite formation inside magnetosomes; Mann, 1983). Biologically induced or passive biomineralization is the dominant mineral-

ization mechanism among bacteria and results from the interaction between the activity of the organisms and the external environment. Bacteria promote mineral precipitation principally through their metabolic effects on system geochemistry with respect to saturation state of potential minerals and/or the kinetics of precipitation reactions. Bacterial promotion of mineral formation—often in association with the bacteria cell, is commonly referred to as authigenic mineral or biomineral precipitation. Biomineral formation is widely described for a broad array of mineral types (e.g., carbonates, sulfides, phosphate minerals, Fe and Mn oxides, uraninites, silicates) and over significant geologic time scales, from almost every environment on earth (Sunda and Huntsman, 1987; Beveridge, 1989; Thompson et al., 1990; Ghiorse and Ehrlich, 1992; Southam and Beveridge, 1992; McLean et al., 1996; Mojzsis et al., 1996; Fortin and Beveridge, 1997; Konhauser, 1998; Beard and Johnson, 1999; Ingebritsen et al., 2000). Biomineralization is currently being evaluated as a potential process that can be harnessed for bioremediation strategies involving solid phase capture of metals and/or radionuclides from contaminated water systems (e.g., Warren et al., 2000) or reduction of metal contaminants (Lovley and Anderson, 2000). Thus, biological influence for metal behaviour extends beyond just surface scavenging capacity, to a direct influence on the types, concentrations and locations of potentially reactive mineral phases.

This bacterial promotion of mineral formation occurs through a variety of geochemical pathways; all ultimately reflecting the inherent thermodynamic constraints on mineral formation. Bacteria cannot change these constraints, they can however, speed up the rate at which those reactions occur, or promote changes in geochemical conditions to those favourable for precipitation to occur, often through influences on redox or pH. For example, microbially mediated metal reduction can lead to precipitation of minerals. Lovley et al. (1991) and Lovley and Phillips (1992) demonstrate that bacterial enzymatic reduction of U(VI) to U(IV) lead to rapid epicellular precipitation of uraninite on the bacterial surface. Bacterial oxidation of Mn(II) to Mn(IV) and subsequent precipitation of Mn oxides has also been widely observed to occur (Nealson et al., 1989, 1999a). It has been suggested (Sunda and Kieber, 1994) that

the oxidation of Mn(II) to Mn(IV) by bacteria promotes abiotic oxidation of HA and FA compounds by the Mn (IV) oxides with a concomitant release of low molecular weight organic compounds that are more readily accessible to the microbes as a carbon source than the more refractory HA and FA. Parmar et al. (2000) show that reduction of ferrihydrite by *S. alga* leads to the precipitation of Fe(II) carbonates (siderite) and associated Sr capture by the Fe(II) carbonate.

Further, metals bound to the bacterial surface can subsequently precipitate through reactions with solution ligands such as S^{2-} , HCO_3^- , CO_3^{2-} , SO_4^{2-} and OH^- , or with metabolic waste products such as H_2S (McLean et al., 1996; Fortin et al., 1997). The precipitates formed generally reflect the chemical composition the environment in which they are growing, and both metal sorption and subsequent mineralization will reflect solution composition of the environment (Konhauser, 1998; Konhauser et al., 1993; Douglas and Beveridge, 1998). Identical minerals are found associated with a variety of physiologically diverse bacteria in the same environment (Lowenstam and Weiner, 1989; Konhauser et al., 1993). This result underscores that the bacterial surface is important as a nucleation template, but that system geochemistry controls the precipitates that form (Konhauser et al., 1993; Warren and Ferris, 1998). Actively metabolizing bacteria pump out protons and metabolic waste products, which can influence the pH and chemical composition of their immediate surroundings. A microenvironment directly surrounding the cell can thus be produced that may be oversaturated in some respect and may induce precipitation of specific mineral types from solution (Ferris, 1997; Fortin et al., 1997; Douglas and Beveridge, 1998). It is important to keep in mind that system geochemistry in the microzone adjacent to bacterial cells can differ among species occurring in the same environment; leading to different minerals being formed in any given cell's vicinity.

Beveridge et al. (1983) examined the low-temperature diagenesis of metals chemically complexed to bacteria (*B. subtilis*; in which the only metal available for diagenesis was bound to the bacterial walls). In an artificial sediment system, they studied the formation of mixed assemblages of crystalline metal phosphates, sulfides and organic condensates. The

results showed that organic polymers of biological origin can play a role in authigenic mineralization and that bacteria do actively nucleate minerals during such processes. Biological materials provided a source of phosphorus for deposition of phosphate minerals and were shown for the first time to be the site of metal sulfide framboid formation.

6.1.1. Characteristics of authigenic minerals

Bacterially induced precipitates can form on the inside, outside or even some distance away from the cells (Ferris, 1997). Metal aggregates deposited within the cell wall itself are physically restricted in size by intermolecular spaces. Only fine-grained precipitates are formed, and these are not easily redissolved or replaced by protons or other metals (Beveridge et al., 1995; Konhauser, 1998). In contrast, epicellular grains reaching several micrometres in diameter can form on the surface of bacterial cells, sheaths and capsules (Beveridge, 1989; Warren and Ferris, 1998). Inorganic grains that are commonly found in a variety of freshwater environments have characteristics including size, crystallinity, chemical composition and spatial orientation that suggest that their origin was authigenic (Konhauser and Urrutia, 1999), and that bacterial mineralization is a widespread and important phenomenon. The grains tend to be amorphous, poorly structured and small in size ($< 1 \mu m$, majority $< 100 nm$) and thus possess high potential geochemical reactivity (Nelson et al., 1999a).

The amorphous nature of precipitates at bacterial surfaces is explained by the "Ostwald Step Rule", which states that the least stable solid phase with the highest solubility will be formed first in a consecutive precipitation reaction. The nucleation of a more soluble phase is kinetically favored as it has a lower solid-solution interfacial tension compared to less soluble or more stable and often more crystalline phases (Stumm, 1992). The initial nuclei of biological mineral precipitates are commonly amorphous or contain highly metastable ratios of both major cations and trace constituents (Mann, 1989; Stumm, 1992).

Bacteria that deposit Fe(III) and Mn(III,IV) oxides in structures outside their cells are ubiquitous and are both Gram-positive and Gram-negative (Mann, 1990). Fe- and Al-silicates formed on the

surface of freshwater bacteria are apparently biomineralized in preference to other minerals that might be supersaturated in the aqueous environment (Konhauser and Urrutia, 1999). TEM analyses commonly indicate bacterial cells partially to completely enclosed within iron-rich epicellular matrices or with intracellular precipitates (Konhauser, 1998). Iron precipitates formed on the surface of three viable, non-metabolically active, strains of bacteria that varied in their surface characteristics (*B. subtilis*, Gram positive, no capsule; *B. licheniformis*, Gram positive, polyglutamic acid capsule; and *P. aeruginosa*, Gram negative, alginate capsule) were identified by selected area electron diffraction, as poorly ordered ferrihydrite on all three bacterial surfaces (Warren and Ferris, 1998). Ferrihydrite has also been shown to be deposited on the organic remains of dead cells, suggesting that iron mineralization can occur independently of cell morphology, classification, or physiological state (Konhauser, 1998). Ghiorse (1984) observed that Mn^{2+} may first bind to acidic polymers on bacterial surfaces, and then be biologically oxidized and subsequently precipitated as Mn oxides. Both Fe^{2+} and Mn^{2+} can be used as energy sources, and there is evidence that a number of enzymatic and non-enzymatic mechanisms may exist, especially for Mn oxidation (Mann, 1990).

Fossilized stromatolites represent ancient microbial-mineral macrostructures. Their counterparts in the modern world are evidenced in the microbial mats commonly found in alkaline freshwater systems. These mineralized biofilms can become significantly large entities (e.g., 40-m-high tower-like structures of microbialite reported from alkaline Lake Van in eastern Anatolia, Turkey; Kempe et al., 1991). Microbial mats with extensive biomineralization often occur in carbonate-rich waters, due to high rates of phototrophic activity by the photosynthetic bacteria driving the mat geochemical dynamics. Thompson et al. (1990) and Thompson and Ferris (1990) evaluated the role of photosynthetic bacteria in a calcareous microbialite (thrombolite) deposit formation in an alkaline freshwater lake. Photosynthetically driven increases in pH were demonstrated to cause gypsum, calcite and magnesite biominerals to form in direct association with the cells, resulting in large, visible structures of carbonate bacterial assemblages.

6.2. Bacterially promoted mineral dissolution

Bacterial activities can also actively promote the dissolution of minerals. For example, a variety of studies have looked at the reductive dissolution of minerals associated with the decomposition of organic matter by bacteria (e.g., Arnold et al., 1988; Stucki et al., 1987). In this instance, bacteria use the mineral as a terminal electron acceptor coupling the oxidation of organic matter to the reduction of mineral metal ions, leading to subsequent dissolution of the mineral. Bacteria can couple a wide array of oxidation redox half reactions to the reduction of metal ions (e.g., a wide variety of organic substrates: formate, lactate, pyruvate, butyrate, toluene, phenol; as well as H_2 , and reduced S compounds; see Lovley, 1995; Stumm and Morgan, 1996; Morel and Hering, 1993).

Lovley (1991, 1995), Lovley and Chapelle (1995) and Lovley et al. (1998) have described the biological process and associated geochemical implications of Fe and Mn oxyhydroxide dissolution due to reductive bacterial action. Such dissolution processes can promote regeneration of metals associated with the solid oxide to the solution phase. Dissimilatory iron and/or manganese reduction is a widespread function across the prokaryotic (bacteria and archaea) phylogenetic tree. It occurs in several functionally different organisms such as anaerobic sulfur reducing bacteria (e.g., *Geobacter metallireducens*) as well as facultative aerobes (e.g., *S. putrefaciens*; Nealson and Saffarini, 1994). It is clear that bacteria do enzymatically reduce both Fe(III) in Fe-oxyhydroxides and Mn(III,IV) in Mn-oxyhydroxides; i.e., a variety of bacteria specifically target these mineral phases to couple to oxidative reactions involving reduced substrates deriving energy from the process to be used for growth (Lovley, 1995). It is evident that reductive dissolution of Fe oxyhydroxide in natural environments is controlled predominantly by microbially mediated processes, as abiotic reductive dissolution reactions are so kinetically slow that despite conditions of anoxia, the oxidized Fe(III) form will persist (Munch and Ottow, 1983; Lovley, 1991, 1995; Parmar et al., 2000). However, it should be noted that not all Fe oxyhydroxides are reduced by microbial action with similar ease. In general, more amorphous Fe oxyhydroxides, e.g., poorly or-

dered ferrihydrite are readily reduced, while more crystalline Fe oxides such as goethite are not (Phillips et al., 1993).

Bacteria also contribute indirectly or passively to the dissolution or solubilization of elements through the production of acids or complexing compounds of low molecular weight, which provide a non-enzymatic dissolution pathway (i.e., there is no direct bacterial action to cause this reaction; nor do they apparently utilize the potential energy from these dissolution reactions). These organic compounds, e.g., carboxylates, have a high affinity for both dissolved cationic species (such as metals) and metals at the surfaces of minerals such as silicates and oxides. For instance, it has been demonstrated that direct attachment of bacteria occurs at mineral surfaces through EPS secretion (Forsythe et al., 1998) and that etching or weathering of mineral surfaces occurs in close proximity of these attached bacteria and especially the EPS secretions (Barker et al., 1998; Rogers et al., 1998; Welch et al., 1999; Welch and Ullman, 1999). This process has been demonstrated for a wide variety of minerals particularly feldspars, and may relate to nutrient limitation. For example, the mineral weathering may provide a biochemical strategy to actively secure limiting nutrients such as phosphorus (Rogers et al., 1998). Rogers et al. (1998) show that bacterial colonization and weathering of feldspars favoured those minerals that had phosphorus inclusions such as apatite. Further, they found heavy colonization and weathering of ammonia-containing orthoclase. In both cases, feldspars that contained no P minerals or orthoclase without ammonia were neither colonized nor weathered.

7. New approaches

7.1. *Molecular biological techniques in microbiology*

One of the most revolutionary techniques that will advance the field of aquatic biogeochemistry is the in situ phylogenetic identification of microbial strains. Prior to the recent advances that have been made in molecular biological techniques, the identification of bacteria was necessarily based on their isolation and cultivation from field samples. This was a lengthy process that is thought to be highly

inaccurate in representing the actual community diversity that was present in the original field sample, and often unsuccessful in culturing the key community players (i.e., culturability does not necessarily equal ecological importance). The inherent anthropocentric view of morphological diversity underlying evolutionary diversity has been completely overturned with the advances in molecular biology and the mounting evidence that the phylogenetic diversity (e.g., functional diversity) of the prokaryotic world exceeds the eukaryotic by orders of magnitude; while ironically appearing morphologically non-diverse.

Traditionally, microbiologists classified microorganisms based on their morphology and nutrient requirements, and techniques such as selective enrichment, pure culture isolation, and most probable number estimates were used to isolate, identify, and enumerate bacterial species. It is now estimated that in many environments, more than 99% of organisms seen microscopically are not cultivatable by standard techniques (Amann et al., 1995). Two reasons why microbes cannot be cultured are that the cultivation conditions may not be suitable for the growth of an unidentified species or that the organisms have entered a non-culturable state (Amann et al., 1995).

The pure-culture view of microbial diversity has, in the last 20 years been shattered, beginning with the work of Woese et al. who, in the early 1980s, reported that naturally occurring organisms could be identified in microbial populations without cultivation (Barns and Nierzwicki-Bauer, 1997). A molecular sequence-based phylogenetic tree established by Carl Woese in 1987 (Pace, 1997) emerged, that could relate all organisms and reconstruct the history of life through comparison of 16 subunit ribosomal RNA (rRNA) sequences (Pace, 1997). The current phylogenetic tree of life is divided into three primary lines of evolutionary descent termed “urkingdoms” or “domains”: Eucarya, Bacteria, and Archeae. Important for this review is that in situ techniques based on the phylogeny (genetic relationship between organisms) are able to give a much better “snapshot” of the microbial diversity in a natural sample. In turn, that microbial diversity may help in interpreting the snapshot of metal behaviour ultimately seen in any given system. The in situ rRNA hybridization technique has allowed not only for the

identification of target microbial species within the larger community of organisms, such as a biofilm, but also their spatial distribution within that community (Santegoeds et al., 1998; Sternberg et al., 1999).

Excellent reviews on the molecular approach to microbial diversity and the *in situ* detection of microbial cells are given by Pace (1997) and Amann et al. (1995), respectively, and more recently summarized in Madsen (2000). The reader is referred to these papers as an in-depth discussion of the development of the rRNA approach and microbial evolutionary diversity is beyond the scope of this review. However, a brief summary is provided, as it is the authors' view that these techniques used in conjunction with other new microscopic and spectroscopic techniques will substantially aid in our challenge to understand the mechanisms controlling metal behaviour. This molecular identification approach using nucleic acid analyses is possible because molecular systematics using ribosomal RNA (discussed subsequently) are the backbone of identification and evolutionary position of life, and techniques to extract nucleic acids directly from environmental samples have been developed (Madsen, 2000).

Nucleic acids (deoxyribonucleic-acid, DNA, ribonucleic-acid, RNA) are biological macromolecules that contain the essential information to conserve and carry out life. Ribosomal RNA (rRNA) is a structural component of ribosomes, which are involved in protein synthesis; thus, the genes that code for rRNA are highly conserved. Ribosomal RNA was chosen as the molecule on which to base the evolutionary divergence of organisms because mutations that lead to changes in ribosome structure are often lethal to bacteria and therefore evolve quite slowly. The 16S rRNA macromolecule was chosen as the most suitable molecule for the purposes of comparison due to its intermediate length (between 5S and 23S) and evolutionarily conserved information (Nealson and Stahl, 1997). Parts of the molecular sequence of nucleotides of rRNA are universally conserved, others show differences. It is the comparison of the relative similarities and differences in these nucleotide sequences that allow the evolutionary similarity among organisms and the overall diversity to be determined.

An average bacterial 16S rRNA molecule has a length of 1500 nucleotides (Amann et al., 1995).

Two key conclusions were made by Amann et al. (1995) based on early results using molecular-sequence-based techniques. The first is that direct rDNA sequences recorded to date have only begun to indicate the extreme diversity in the two domains that make up the prokaryotes: the Archaea and the Bacteria. The best guess at the present time, is that we known perhaps 10% of the diversity that occurs in nature in the prokaryotic world; of that 10% less than 1% is in culture. The enormous implication of these figures is that the microbial world is infinitesimally more diverse than that of the eukaryotic world; and is nowhere near being described, catalogued or understood in terms of global geosphere implications. And secondly, the authors note that only with *in situ* investigation of microorganisms, i.e., organisms within their natural microenvironments, will the spatial and ecological distribution of organisms and their function be determined effectively. This is possible with the fluorescence *in situ* hybridization (FISH) technique.

Fluorescence *in situ* hybridization involves applying fluorescently monolabeled DNA probes to environmental samples. These oligonucleotide probes have been designed to be complementary to, and hybridize with, target rRNA within intact/whole fixed cells (Nealson and Stahl, 1997). A brief outline of the approach taken for a complex microbial consortia, such as that observed in a biofilm is given to illustrate the utility of the approach. In complex samples such as biofilms, where it can be expected that a large number of different groups, genera, and species of bacteria will exist, a "top-to-bottom approach" (Amann et al., 1995) in phylogenetic identification is taken using FISH. In this approach, probes designed for phylogenetic domains, the Archaea, Bacteria and Eucarya, are applied first to an environmental sample to identify the major groups that may be present. Once the major groups have been identified, more specific probes to identify certain genera can then be applied. If, for example, the organisms of interest are in the domain of Bacteria, more specific probes can then be applied to the sample to target narrower taxonomic levels (groups, general, species) by using probes that have been developed to target the more variable regions of the 16S rRNA sequences of specific organisms. The FISH technique has several advantages; the most obvious be-

ing a phylogenetic characterization of the microbial consortia within a complex environmental sample. Moreover, FISH allows a high resolution of both the spatial distribution and abundance of target organisms (Amann et al., 1995; Nealson and Stahl, 1997); and may identify organisms within the sample that have previously been unidentified or uncultured. In addition, Amann et al. (1995) indicate that quantification of the probe signal can be related to the in situ growth rates of target cells. Raskin et al. (1995) successfully related the cellular rRNA content to the growth rate of a previously unknown organism (*Desulfovibrio vulgaris*, strain PT-2). The growth rate of the bacteria was found to be higher in young biofilms compared to more mature biofilms.

It should be kept in mind that the field of environmental molecular microbiology is fairly recent and thus there are some limitations to the approach; reflecting more the gaps in our knowledge base rather than limitations with the FISH technique. The phylogeny of the organisms within the sample can be characterized as long as there is a similar genetic sequence in the databank. However, knowing the phylogeny of that organism does not necessarily reveal its ecological function. It is common for bacteria from several different phylogenetic groups to have the same metabolic function. Further, the same strain of bacteria can shift its metabolic function dependent on system conditions, for example, from sulphate reduction to iron reduction. Unless the targeted organism has been cultured, or the rDNA sequence of that organism is found to be very similar to a previously cultured organism, the activity of that organism can only be inferred (Barns and Nierzwicki-Bauer, 1997). As of yet, the metabolic functions of the various branches of the phylogenetic tree are only loosely and incompletely outlined. However, we believe that combined approaches where the application of such in situ molecular identification techniques as FISH, in concert with more traditional metal geochemical characterization are essential to the understanding of the biogeochemical linkages and processes controlling trace metal behaviour.

7.2. Microsensor techniques

Microsensors or microelectrodes can be used to measure in situ profiles of specific chemical species

within biofilms or sediments at high spatial resolution. Revsbech (1989, in de Beer and Schramm, 1999) developed the first microsensor for profiling of dissolved oxygen in sediments and biofilms. Microsensors now exist that measure $\Sigma\text{H}_2\text{S}$, pH, N_2O , NO_2^- , NO_3^- , NH_4^+ , CH_4^+ , CO_2 , glucose, light, flow and diffusion (individual developers referenced in Lens et al., 1995; Santegoeds et al., 1998; de Beer and Schramm, 1999). The high spatial resolution ($< 50\text{ }\mu\text{m}$) of the microsensors is due to the small size of the tip diameter, 1–20 μm (Santegoeds et al., 1998). The small size and analyte consumption of the microsensors allows the sample to be probed without disturbing either the structural/ecological distribution within the biofilm or the chemical gradients (Santegoeds et al., 1998). A good introduction to the various types and functions of microsensors is given in Santegoeds et al. (1998), with descriptions of electrochemical, optical, amperometric microsensors and micro-biosensors. These new microsensors permit accurate characterization of geochemical conditions occurring within localized, geochemically intense zones such as redox clines in sediments and biofilms. The high spatial resolution of the geochemical information afforded by these microsensors will aid to quantitatively understand the processes involved in metal dynamics.

7.3. Microscopic techniques

One of the major challenges for environmental geochemists is the accurate prediction of metal behaviour in aquatic environments. The inherent complexity of natural systems and the specificity of sorbent–sorbate reactions underscores the absolute requirement for a mechanistic understanding of interfacial region processes. In particular, the molecular geochemistry occurring at surfaces can neither be predicted based on macroscopic information nor understood without some identification of the controls on the processes that occur in this region. Recently developed and increasingly applied microscopic techniques for the visualization of interfacial regions of solid surfaces are confocal laser scanning microscopy (CLSM), environmental scanning electron microscopy (ESEM), and atomic force microscopy AFM (Nealson and Stahl, 1997; Barns and Nierzwicki-Bauer, 1997; Eggleston et al., 1998),

which when used in conjunction particularly with spectroscopic information (e.g., X-ray absorption spectroscopy, XAS; discussed subsequently) can provide both visual and mechanistic information.

All three imaging or microscopic techniques allow for visualization of hydrated samples (Barns and Nierzwicki-Bauer, 1997) while sample preparation for the more classical electron microscopic techniques (SEM and TEM) can destroy fragile biological samples such as biofilm architecture. With ESEM, and attached X-ray energy dispersive analyses (EDS) samples such as hydrated microbes can be viewed and quantitative elemental analyses undertaken under ambient atmospheric conditions, without needing a vacuum and gold coating (e.g., Timofeeff et al., 2000). Scanning probe microscopy (e.g., AFM) allows interface to be imaged often at the atomic scale, thereby offering superior resolution. The ability to image interfacial regions of hydrated, nonprocessed samples, has obvious interest to environmental metal geochemistry (e.g., NOM attachment to mineral surfaces, mineral dissolution or growth; Maurice and Lower, 1998; Plaschke et al., 1999a,b).

For example, three-dimensional reconstruction of biofilm architecture is possible with CLSM as the sample can be optically sectioned vertically and horizontally without having to slice the sample, as out of focus planes are excluded from the image (Brown et al., 1998; Manz et al., 1999). Lawrence et al. (1995) have extensively investigated microbial spatial relationships within biofilms using CLSM coupled with fluorescent probes. In a study of river water biofilms, Neu and Lawrence (1997) used the LIVE/DEAD stain (Molecular Probes, Eugene, OR, USA) to quantify the numbers of the viable and nonviable bacteria in the community. A non-specific nucleic acid stain was employed, as well as a number of lectin stains for specific carbohydrates known to be components of the EPS of certain bacteria. Over time, the bacterial biomass distribution within the biofilm changed and heterogeneity with regards to certain bacteria and their associated EPS was observed. Lawrence et al. (1998) later used a “three” channel approach to characterize the two- and three-dimensional distribution of algal, bacterial, and polymer biomass and biofilm thickness of river water biofilms. Bacteria were stained with a fluorescent nucleic acid stain, the exopolymer with a lectin probe, and the algal cells

were visualized with autofluorescence. Manz et al. (1999) used the FISH technique in conjunction with CSLM and digital image analysis. With this approach, the authors could characterize a change in the dominant bacterial groups in the larger biofilm community over time. Inverted light microscopy in conjunction with the FISH technique and digital data analysis has also been shown to give a good visual description of biofilm structure (Lewandowski et al., 1999). Thus, it is possible, with these techniques to obtain morphological, quantitative, identification of cell surface components (via labelling), identification of specific species within microbial consortia, distinctions between viable and non-viable cells, and spatial organization of microorganisms occurring within different environmental matrices (Barns and Nierzwicki-Bauer, 1997).

7.4. Spectroscopic techniques

A variety of surface spectroscopy techniques such as low energy electron diffraction (LEED), photoelectron (UPS, XPS), and Auger provide detailed geometric and electronic information on solid surfaces. These techniques have been applied to both mineral and bacterial surfaces with some success. One drawback of these electron spectroscopies is that they require a vacuum, and thus processes occurring at the solid solution interface cannot be studied in situ. In contrast, X-ray absorption spectroscopy (XAS) does allow the in situ examination of samples and has been used extensively to look at a variety of mineral surfaces (e.g., Brown et al., 1989; Chisholm-Brause et al., 1990; Peterson et al., 1997; Parkman et al., 1998). These techniques are reviewed in detail by Brown (1990), Brown et al. (1999) and O'Day (1999). XAS spectroscopies provide information on the molecular processes occurring at surfaces and give quantitative insight into speciation and the types of bonds being formed. The reader is referred to O'Day (1999) for an excellent review of these techniques. The purpose of this review is to highlight their potential utility for providing relevant information for processes important in understanding metal dynamics.

Two commonly used, complementary XAS spectroscopic techniques are X-ray or extended X-ray absorption fine structure (XAFS-EXAFS; informa-

tion on interatomic distances) and X-ray absorption near-edge structure (XANES; information on coordination geometry). These techniques provide chemical speciation or bonding information occurring between specific surface groups and sorbing ions. These approaches are being used with success to identify the heterogeneous nature of surfaces (e.g., Stipp et al., 1992), the types of reactions occurring between various metal and mineral or organic surfaces (e.g., Reeder et al., 1994; Sturchio et al., 1997; Xia et al., 1997a,b), as well as mechanisms of mineral reductive dissolution (e.g., Eggleston et al., 1996; Nesbitt et al., 1998). EXAFS and XANES techniques are especially relevant to metal studies as they are element specific and can provide information on metal speciation, e.g., speciation of organically bound sulfur in marine sediments (Vairavamurthy et al., 1994), Pb-humic complexes (Manceau et al., 1996), and metal partitioning between solution and solid phases (O'Day et al., 1998; Carroll et al., 1998). The types of molecular scale information on surface reactions provided by these techniques start to explain mechanistically the metal-sorbent specificity seen in sorption reactions. These types of studies will provide the essential information required to constrain macroscale models of metal dynamics within natural aquatic environments.

8. Conclusions

The inherent complexity, heterogeneity and ever-changing relative importance, of the factors controlling metal dynamics in freshwater environments precludes the development of a simplistic model of metal behaviour. Factors important in controlling metal speciation, solid–solution partitioning, type of solid phase association whether as a surface complex or precipitate, will ultimately determine transport, reactivity and fate of metal contaminants. From the developing framework, it is apparent that these factors cover a broad spectrum of traditionally segregated scientific disciplines, and the interplay between such factors is likely to be of extreme importance in determining the location, extent and rate of processes important in metal cycling. In particular, metal reactions occurring in natural environments are likely to be controlled by a suite of linked physical, chemical

and microbiological processes. Thus, identification and understanding of these linkages will provide key insights into the prevailing controls on metal behaviour in different systems. In particular, the fields of surface chemistry, environmental microbiology and geochemistry are implicit in any relevant paradigm.

A mechanistic understanding of metal dynamics requires that processes are evaluated in an integrated cross-disciplinary fashion. Further, the relationship between macroscale or system geochemistry and microscale or localized conditions has to be more clearly delineated and characterized. It is evident that bulk system geochemistry will not adequately describe conditions occurring within localized, intense geochemically reactive zones such as interfacial regions, biofilms and sedimentary redox microclines. Further, quantitative information on the nature, extent and controls of reactions occurring at the microscale will better constrain our geochemical models of metal behaviour in aquatic systems. The field of environmental geochemistry has made huge advances in the last 20 years in understanding the likely controlling factors at the macroscopic scale. The relevance of environmental microbiology is more and more appreciated and, more importantly, the focus of increasing numbers of quantitative investigations. Modelling approaches are beginning to converge on a unified understanding of surface reactions and the inclusion of bacterially promoted reactions is gaining more widespread attention.

New advances in molecular biological techniques are revolutionizing the field of environmental microbiology and particularly our ability to identify microbes *in situ* without cultivation. Further, new spectroscopic and microscopic techniques are providing more detailed information at the microscopic scale; essential if we are ever to untangle the complexities of metal specificity in surface reactions; and more importantly make the links back to modelling system dynamics. New microsensor probes that permit geochemical characterization at much higher spatially resolved scales (e.g., tens of microns) allow for determination of geochemical conditions within the geochemically relevant locations where key reactions are taking place. These new techniques, used in concert, provide a powerful set of tools that will allow far more accurate and detailed understanding

of the biogeochemical processes and controls that determine the snapshots of metal behaviour we observe in natural environments.

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