Characterizing Clay-Microbe-Metal Interactions: Implications for Metal Immobilization

Characterizing Clay-Microbe-Metal Interactions: Implications for Metal Immobilization

By

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Abstract

Bentonite clays and Fe-reducing bacteria have been identified independently of one another as important geochemical agents with the ability to influence metal and metalloid mobility in aqueous environments. In the context of metal(loid) immobilization, however, the numerous interactions occurring between Fe-reducing bacteria and bentonite clays and clay mineral constituents remain largely undescribed. This thesis examines the immobilization of two cationic metals (Cu^{2+} and Cd^{2+}) and an oxyanion-forming metalloid (As^{5+}) from aqueous solution by natural bentonite clays, and compares sorption to the sterile bentonite clays with sorption in the presence of two anthropogenically-influenced Fe-reducing bacterial enrichments. The two main research questions under investigation in this thesis are: 1) What are the influences of potentially metal-tolerant Fe-reducing bacteria on metal(loid) uptake by bentonite clay sorbents of varying smectite content and on the stability of bentonite clay-sorbed metals and metalloids?; and 2) Are there differences in the influence of Fe-reducing bacteria on Cd^{2+} and Cu^{2+} sorption to bentonite clays when As^{5+}, a reducible, metabolically-available metalloid, is present in solution?

Experimental results herein demonstrate the importance of a number of *interconnected* processes that together influence solid- and solution-phase chemistry (e.g. pH). These processes include mineral and amorphous phase dissolution (e.g. calcite, dolomite, smectite, chlorite, illite, amorphous Fe-oxyhydroxides), precipitation of amorphous phase material (e.g. Fe-oxyhydroxides), sorption/desorption reactions, complexation with dissolved organic carbon, and redox reactions involving solution- and solid-phase species (e.g. reductive dissolution of amorphous Fe-oxyhydroxides coupled to the oxidation of acetate, aerobic oxidation of acetate, reduction of arsenate coupled to the oxidation of acetate). A number of these processes were enhanced or facilitated by the presence and activity of Fe-reducing bacterial enrichments, inherently demonstrating the importance of bacterially-driven processes in controlling solid- and solution-phase chemistry, including the partitioning of toxic trace elements between solid and solution phases.

While the influence of bacterial augmentation on Cu^{2+} sorption was negligible in these experiments, uptake of Cd^{2+} was enhanced in the presence of Fe-reducing bacteria, and perhaps most profoundly, As displayed time-dependent desorption during the experimental timeframe in the presence of Fe-reducing bacteria. These results highlight the existence of potential limitations to the use of bentonite clay sorbents for metal(loid)-contaminated wastewater reclamation, identifying the microbially-vulnerable nature of metal(loid) sorption reactions, and further supporting that the long-term stability of sorbed metal(loid)s is questionable in the presence of Fe-reducing bacteria. Abundant facets concerning clay-bacterial-metal(loid) interactions in the environment exist which require further in-depth characterization and experimentation to generate deeper understanding of the potential utility of, as well as limitations to, the use of clay and clay mineral sorbents for the sequestration of toxic metals and metalloids from aqueous solution.

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1.0 Introduction

Clay Mineral-Microbe-Metal Interactions in the Environment: From Science to Application

Submitted by Steven P. Holland and Lesley A. Warren
to *Earth-Science Reviews*

Abstract

Metal contamination of freshwater systems remains an issue of critical importance at a global scale. Both clay minerals and microorganisms have received increasing recognition as important geochemical agents with the ability to impact metal behaviour in environmental systems. The adsorptive characteristics of clay minerals, attributed primarily to their high surface area, cation exchange capacity, and net negative particle charge have been well documented, and clays have found substantial utility in various environmental applications (e.g. water treatment, landfill liner construction). Similarly, microorganisms are increasingly appreciated to influence contaminant behaviour through numerous active and passive processes including generation of microenvironments, impacts on metal solubility, and mineral alteration, formation and dissolution. However, the implications of microbial activity in clay mineral-metal interactions are not well described, despite their likely importance. While abundant research has targeted the characterization of metal immobilization either by microbes or by clay minerals, as well as microbial influences on clay minerals (e.g. mineral dissolution and precipitation, influences on suspended particle flocculation, and alteration of clay minerals), to date, minimal literature exists which specifically assesses microbial-clay mineral interactions in the context of metal immobilization. As molecular based approaches specifically targeting DNA extraction from clay matrices have been developed, emerging evidence has indicated a greater abundance and diversity of endemic clay microbial communities than previously anticipated, highlighting real opportunities to advance the development of more effective bio-based clay mineral water treatment strategies if their impacts are more fully constrained. Scientific understanding in this critical area would identify novel microbially-associated opportunities for the development of more effective and sustainable clay mineral-based environmental applications, as well as contributing fundamental new insights into important environmental biogeochemical processes. This review is not intended as an exhaustive review of clay mineralogy, environmental microbiology or trace element biogeochemistry; rather, this review provides a synopsis of the current scientific literature identifying emerging evidence and current gaps in understanding specifically focused on: a) clay mineral-metal interactions; b) potential impacts of microbial-clay mineral interactions in the context of metal sequestration; c) environmental microbiology of clays; d) new and emerging technologies for the characterization of clay-endemic microbes and interactions between these communities and clay substrates; and e) clay mineral-based water treatment approaches.

Keywords: sorption, metals, clays, clay minerals, bacteria, water treatment, molecular based characterization techniques

1.1 Review Scope

This review will specifically focus on clay mineral adsorbents, environmentally ubiquitous and abundant materials that possess many characteristics which make them effective cationic metal adsorbents. To date, clay minerals have received less attention than oxide minerals and organics with regard to metal adsorption. Further, while emerging evidence indicates diverse microbial associations with clays are likely more common than previously thought, bearing potential implications for clay mineral-metal interactions, minimal characterization of microbial-clay mineral interactions with respect to clay mineral-metal sequestration has occurred to date. While not intended to be an exclusive, in-depth treatise on clay mineralogy or exhaustive methodological review of techniques applicable to the characterization of environmental microbes, this review provides an overview of the current literature specifically focused on and pertinent to clay mineral-microbe-metal interactions in the environment. It includes fundamental aspects of relevant surface chemistry applicable to clay mineral-metal adsorption, as well as pertinent information concerning clay mineral-metal interactions, microbial-clay mineral interactions and microbial-metal interactions, identifying the use of new and emerging technologies and gaps in current understanding.

1.2 Relevance: Metal Contaminants in the Environment

1.2.1 Impacts and Important Controls on Metal Environmental Behaviour

The accelerated increase in metal concentrations in aqueous environments is associated with a variety of anthropogenic activities (e.g. casting, mining, refining, smelting, metal coating, ammunition production, paint manufacture, battery production, tannery operation, electroplating), which collectively contribute substantive quantities of metals to aqueous systems worldwide (Leung et al., 2000, Singh & Cameotra, 2004, Potgieter et al., 2006, Veli & Alyüz, 2007, Chalermyanont et al., 2009, Ijagbemi et al., 2009). Metal contaminants impose a significant threat upon exposed ecosystems and humans alike, and those of particular environmental significance include Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo, Ag, Cd, and Hg (Leung et al., 2000). Several of these so-called “heavy metals” are essential to biological processes in minute concentrations, but demonstrate extreme toxicity when present at elevated concentrations often associated with industrial discharges; note that, despite its prevalence in the literature, the term “heavy metal” will not be used in this review, due to discrepancies in the term’s meaning (see Duffus, 2002 for details) (Kosolapov et al., 2004, Malik, 2004, Abollino et al., 2008). Copper, for instance, is an essential trace element in all living organisms, which functions in mammals as an electron acceptor or donor within metalloenzymes; exposure to higher doses of Cu, however, negatively affects the liver, kidneys, central nervous system, immune system, and bone structure in a variety of detrimental ways (Stern et al., 2007). In general, exposure to elevated metal concentrations interferes with the central nervous and haematopoietic systems, impairs higher cognitive function, and causes liver and kidney damage, birth defects, skin lesions, and various types of cancer in humans (Singh & Cameotra, 2004, Florea & Büsselberg, 2006).

Solution speciation is an important determinant of metal behaviour in environmental systems, controlling numerous properties of the metallic contaminant, including toxicity, reactivity, solubility, mobility, bioavailability, persistence, and bioaccumulation (Hill, 1997, Brown et al., 1999, Sparks, 2005). The speciation of metals in aqueous environments is regulated by several factors, including pH, Eh, microbial activity (e.g. via metabolically-coupled oxidation or reduction), and the potential of the particular metal to undergo processes such as photoreduction (Hill, 1997, Lovley & Coates, 1997, Barkay & Schaefer, 2001). Related to speciation, toxicity of metals in the environment is particularly problematic from a remediation perspective, as, unlike many organic compounds, metals are not rendered harmless by biodegradation. Rather, they are persistent and are characterized by a tendency to accumulate in the environment and in the tissues of organisms, thereafter allowing metal contaminants to impose deleterious consequences upon associated food webs and ultimately, human health (Warren & Haack, 2001, Singh & Cameotra, 2004, Veli & Alyüz, 2007, Akar et al., 2009, Ijagbemi et al., 2009). The increased toxicity typically associated with free metal forms in the environment underlines the importance of partitioning between solid and aqueous phases as the dominant control governing metal risk to environmental and human health. Partitioning in aqueous systems is driven by the interactive characteristics of the metals of concern (e.g. speciation, concentration), the aqueous media (e.g. pH, Eh, presence and activity of microorganisms, ligands, competing ions, anions, chelating molecules), and available solid substrates (e.g. Fe- and Mn-oxyhydroxides, zeolites, activated carbon, zero-valent Fe, clay minerals) for metal sorption (e.g. surface charge, surface area, cation exchange capacity, type and abundance of surface sites, microtopography).

1.2.2 Cationic Metal Sorption

Sorption is a general term referring to the accumulation of a substance at a surface or interface and is used without specification of a particular mechanism of uptake or when numerous uptake mechanisms are suspected to occur simultaneously; it includes processes such as adsorption, the two-dimensional accumulation of adsorbate molecules or ions at the external surface of the adsorbent, absorption, in which the sorbate is taken into the internal pore space of the sorbent, and surface precipitation, the three-dimensional accumulation of sorbate at the external surface of the sorbent (Haack et al., 2008, Maurice, 2009). Adsorption processes are of particular importance to clay mineral-metal interactions, and thus this review will focus primarily on adsorption when discussing metal immobilization by clay minerals.

Surface adsorption reactions at solid surfaces play a key role in metal partitioning in environmental systems. Atoms at the surface of a mineral substrate are not fully coordinated, which thus induces the establishment of a different bonding environment for surface atoms as compared to that observed for atoms within the bulk solid; this facilitates the establishment of a dynamic reactive environment at the interface between a mineral and surrounding aqueous solution. Furthermore, mineral surfaces demonstrate heterogeneity in composition and structure, and this heterogeneity ultimately determines the specific surface reactivity of a given substrate; in this regard, a definite distinction exists between the surface and the bulk solid. The lack of full coordination of surface atoms results in higher energy observed at the mineral surface compared to that of the bulk solid, and this surface/interfacial free energy, derived from contributions of van der Waals forces and H-bonding, can be minimized via adsorption of ions to surface sites. A variety of hydroxyl sites exist at a mineral surface in aqueous media, including $>M\text{-}OH$, $>M\text{-}O^-$, and $>M\text{-}OH_2^+$ (whereby >M represents a metal cation at the mineral surface), which undergo surface protonation/deprotonation hydrolysis reactions as a function of solution pH. These hydroxyl sites behave as Lewis bases when deprotonated, and can further function as potential sites for metal cation adsorption; surface complexation between a hydroxyl site and a divalent cation (e.g. Cu^{2+}) can be described by: $S - OH + Cu^{2+} \rightarrow S - OCu^+ + H^+$ (monodentate); or $S - OH + Cu^{2+} \rightarrow (S - O)_2Cu + 2H^+$ (bidentate). Aside from controlling the partitioning of ions between solid and aqueous phases, adsorption is an important process affecting the electrostatic properties of suspended particulate matter, the transport of chemical substances between different reservoirs, and the surface reactivity of solid particles. Cationic metal adsorption occurs via formation of inner-sphere and outer-sphere surface complexes, and incorporation of ions within the diffuse-ion swarm (described in the paragraphs to follow; see Figure 1.1). (Sposito, 1989, Stumm & Morgan, 1996, Maurice, 2009).

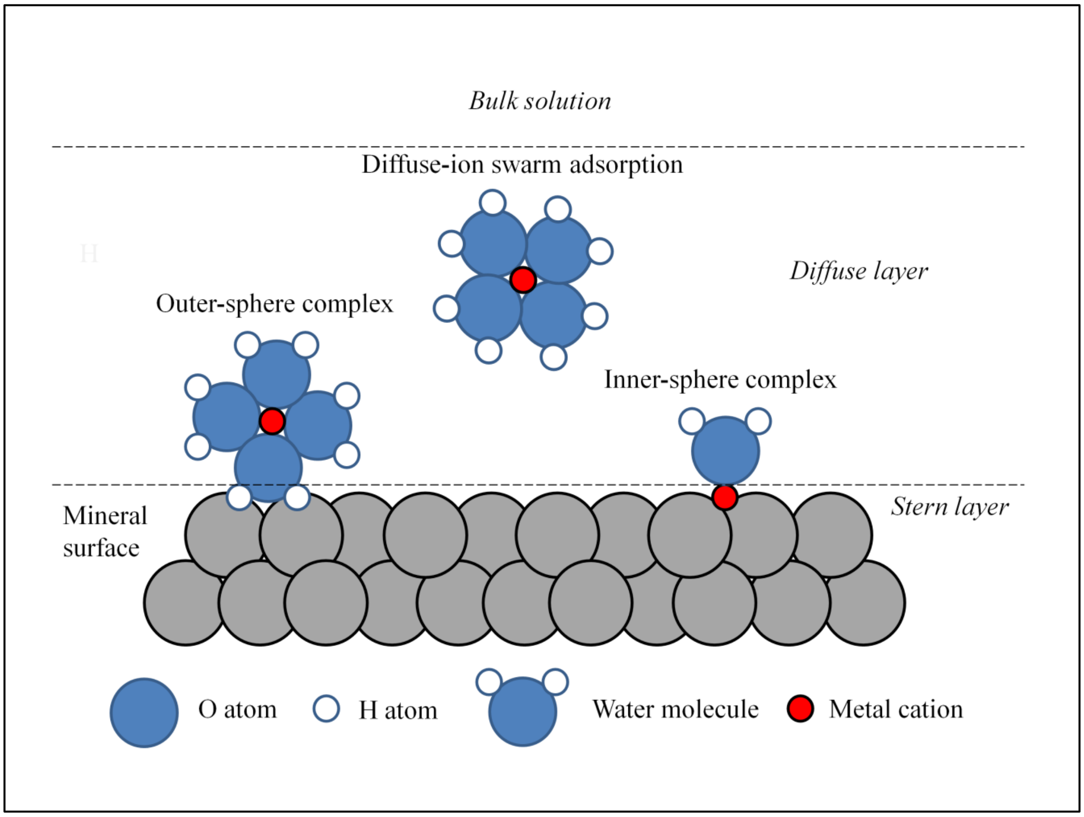

Fig. 1.1 – Schematic of cationic metal adsorption mechanisms. Inner-sphere complexes form when metal cations are directly bound (covalently, ionically) to surface functional group(s) on the adsorbent (note that the cation may remain partially hydrated, but bonding occurs directly between surface functional group and the metal cation). Outer-sphere complexes are hydrated metal cations bound electrostatically to the surface with minimally one water molecule present between the cation and surface functional group. Incorporation into the diffuse-ion swarm involves delocalized electrostatic attraction between the hydrated cation and surface functional groups (i.e. involving no distinct interactions between particular functional groups and the cation; rather, the cation is attracted to the bulk electrical charge of the surface). Modified from Sposito (1989), Stumm & Morgan (1996), and Maurice (2009).

Inner-sphere surface complexation (i.e. specific adsorption) involves the loss of hydrating water molecules from a solvated metal cation, which allows the cation to bond directly via covalent or ionic bond(s) to the participating functional group(s) on the surface of the adsorbent (Sposito, 1989, Stumm & Morgan, 1996, Brown et al., 1999, Sposito et al., 1999, Konhauser, 2007, Maurice, 2009). Electron configuration of the adsorbate ion and the surface functional group(s) is extremely important in controlling inner-sphere surface complex formation (Sposito, 1989). Due to the strong nature of the involved chemical bonding, inner-sphere complexes are relatively stable, and large fluctuations in pH or ionic strength are typically required to facilitate the remobilization/desorption of metals bound via inner-sphere complexation (Brown et al., 1999, Small et al., 2001). The relative strength of inner-sphere surface complexes is dependent upon the number of bonds formed between the complexing ion and the adsorbent surface, increasing in stability from monodentate complexes through bidentate complexes to tridentate complexes (Brown et al., 1999). Adsorption of weakly hydrating metals (i.e. those that readily lose waters of hydration, such as K^+, Cs^+, and many trace metals) to mineral surfaces tends to occur via inner-sphere complexation, with divalent trace metal adsorption affinities coinciding approximately with the Irving-Williams order (i.e. $Cu^{2+} > Ni^{2+} > Co^{2+} > Fe^{2+} > Mn^{2+}$). This adsorption affinity trend is also approximately followed for ligand exchange, and is a reflection of ionic radius (inherently coupled to increasing Lewis acidity character and strength of metal-ligand bond), increased stability coinciding with ligand field stabilization energy, and decreasing effective nuclear charge along the series order. The affinity of an *adsorbate ion* for a given *adsorbent surface* generally increases with the ability of the cation to form inner-sphere complexes, which in turn is dependent upon cationic radius. This phenomenon is attributed to the tendency of cations with larger radii to create a smaller electric field (thus decreasing the tendency to remain solvated in the presence of surface functional groups), as well as to polarize in the presence of charged surface functional groups. However, when considering the adsorption of transition metals, adsorption affinity also strongly reflects electron configuration. (Sposito, 1989, Maurice, 2009).

Weaker, outer-sphere surface complexation via electrostatic attractive forces results when the complexed metal cation is surrounded by a sheath of hydrating water molecules (minimally, one water molecule), which prevents direct chemical bonding between the solvated metal cation and involved functional group(s) on the adsorbent surface (Sposito, 1989, Stumm & Morgan, 1996, Brown et al., 1999, Sposito et al., 1999, Konhauser, 2007, Maurice, 2009). The point of zero charge (pH_{pzc}) of a mineral surface refers to the pH value at which the net surface charge (i.e. the difference between positively and negatively charged surface functional groups) is zero, and is extremely important in the context of outer-sphere complexation (Sposito, 1989, Stumm & Morgan, 1996, Maurice, 2009). Cationic metals (i.e. not including those metals and metalloids that exist as oxyanions in aqueous solution) can only form outer-sphere complexes above the pH_{pzc} of the adsorbent surface, when the surface exhibits a negative charge; outer-sphere complexes are therefore sensitive to changes in pH and ionic strength, permitting the ready remobilization of outer-spherically complexed metal cations upon fluctuation in these solution parameters (Stumm & Morgan, 1996, Brown et al., 1999, Small et al., 2001, Maurice, 2009). Strongly hydrating ions, such as Ca^{2+} and Mg^{2+}, tend to form outer-sphere complexes with negatively charged mineral surfaces (Maurice, 2009). Formation of outer-sphere surface complexes largely depends upon the valence state of adsorbate ions such that trivalent cations are often favoured over divalent cations (Sposito, 1989).

Lastly, adsorption of cations via incorporation into the diffuse-ion swarm is accomplished without requiring the formation of a complex with surface functional group(s). Ions in the diffuse-ion swarm act to neutralize surface charge while remaining delocalized and fully dissociated from surface functional groups, thereby allowing free mobility of the ions in solution. The adsorption of cationic metals into the diffuse-ion swarm is primarily a function of valence and overall surface charge. Similarly to outer-sphere complexation, adsorption of ions via incorporation into the diffuse-ion swarm strictly involves electrostatic bonding. Thus, outer-sphere complexation and incorporation into the diffuse-ion swarm are both non-specific mechanisms of adsorption, which refers to the weak, charge-driven involvement of adsorbate ion and surface functional group electron configurations in controlling adsorption by these mechanisms. The relative strength of adsorption interaction is such that inner-sphere complexes > outer-sphere complexes > diffuse-ion swarm incorporation. In general, cationic metal adsorption tends to follow an adsorption edge, characterized by sharply increased adsorption as pH approaches and exceeds the pH_{PZC} of zero point of charge of a given surface. (Sposito, 1989, Maurice, 2009).

The ability of clay minerals to immobilize metal cations has been demonstrated across a broad range of geochemical conditions, with work to date supporting clay mineral adsorption of numerous metals and metalloids, including Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Cd, and Pb (e.g. Suraj et al., 1998, Chen et al., 2007, Bhattacharyya & Gupta, 2008a, Eloussaief et al., 2009). The terms clay and clay mineral are often used interchangeably, but two distinct definitions exist; a clay is defined as a sedimentary material of ≤2μm particle diameter, while a clay mineral is a fine grained phyllosilicate (i.e. sheet silicate) mineral composed of hydrous aluminosilicate sheets (Maurice, 2009). In general, clay deposits are composed of clay minerals and clay-sized fragments of other mineral constituents including quartz, metal oxides and carbonates (Bhattacharyya & Gupta, 2008a). The layered structure of clay minerals results in large surface area, high cation exchange capacity (CEC), high chemical and mechanical stability, and net negative surface charge over the range of pH values typically observed in aquatic environments; these properties allow clay minerals to attract and adsorb various species of metal cations (Lothenbach et al., 1997, Cooper et al., 2002, Kugler et al., 2002, Usman et al., 2005, Gu & Evans, 2007, Kubilay et al., 2007, Veli & Alyüz, 2007, Abollino et al., 2008, Bhattacharyya & Gupta, 2008a,b, Akar et al, 2009, Ijagbemi et al., 2009, Sipos et al., 2009). Adsorptive properties of clay minerals are largely a reflection of their net negative charge, attributed to isomorphic substitution in tetrahedral and octahedral layers (see section 3.1), and pH-dependent charge, as well as the high CEC (for description of cation exchange, see section 3.2) demonstrated by some clay minerals. As a result of these characteristics, clay minerals encourage metal uptake via inner-sphere and outer-sphere surface complexation with surface hydroxyl sites (e.g. aluminol, >Al-OH, and silanol, >Si-OH), via incorporation of solution ions into the diffuse-ion swarm, and via the exchange of outer-spherically and diffuse-ion swarm-adsorbed cations with ions from solution (e.g. Sposito, 1989, Stumm & Morgan, 1996, Maurice, 2009).

Adsorption has gained popularity as an applied metal sequestration approach, and the ideal adsorbent is characterized by exhibiting several key attributes. Exceptional adsorbents should: a) be inexpensive and readily available; b) demonstrate strong affinity for target metal species; c) be effective adsorbents across a broad range of geochemical conditions and metal concentrations; d) function as an adsorbent with little to no required pre-treatment; and e) demonstrate potential for regeneration (e.g. Prasad & Saxena, 2004, Kubilay et al., 2007). The varying abilities of numerous substrates, including activated carbon, Fe- and Mn-oxyhydroxides, organic matter, and to a lesser extent, clay minerals, to adsorb cationic metals have been documented (e.g. Lothenbach et al., 1997, Suraj et al., 1998, Papini et al., 2001, Bostick et al., 2002, Cooper et al., 2002, Babel & Kurniawan, 2003, Chantawong et al., 2003, Yavuz et al., 2003, Strawn et al., 2004, Usman et al., 2005, Adebowale et al., 2006, Dal Bosco et al., 2006, Potgieter et al., 2006, Abollino et al., 2007, Carvalho et al., 2007, Kubilay et al., 2007, Veli & Alyüz, 2007, Abollino et al., 2008, Bedoui et al., 2008, Bhattacharyya & Gupta, 2008a,b, Chaari et al., 2008, Ribeiro et al., 2008, Ijagbemi et al., 2009). However, many passive remediation technologies applicable for metallic wastewater treatment are biologically-based and are thus dependent upon the activity of microorganisms (e.g. Neculita et al., 2007). The long-term effectiveness of clay mineral-based adsorbent systems, particularly the stability of adsorbed metals, may similarly prove to be affected by microbial activity, thereby indicating a greater need to understand metal-clay mineral-microbe interactions in general.

1.3 Clay Mineral Sorptive Properties

1.3.1 Clay Mineral Structure

Clay mineralogy is a well established field with numerous publications dedicated in their entirety to the classification and laboratory identification of clay minerals (e.g. Grim, 1953, Paterson & Swaffield, 1994 and others from the same volume, Velde, 1995 and others from the same volume, Moore & Reynolds Jr., 1997, Meunier, 2005, Meunier, 2006). The purpose of this review is not to provide a detailed mineralogical investigation of clay minerals; rather, the scope is focused on clay mineral-metal-microbial interactions and thus critical mineralogical information pertinent to clay mineral-metal interactions, particularly concerning adsorption, is provided. Clay minerals contain two distinct sheet types, tetrahedral and octahedral, that stack vertically on top of one another in different arrangements, which forms the foundation for the classification of clay minerals (discussed in the next paragraph). Tetrahedral sheets contain cations (Si^{4+}, Al^{3+}, Fe^{3+}) in tetrahedral coordination with three basal O^{2-} atoms and one unshared apical O^{2-} atom (radius ratio of cation/anion = 0.225-0.414). The tetrahedra are arranged in a six-fold ring pattern such that the three basal oxygen atoms of each tetrahedron are shared with neighbouring tetrahedral groups in the ring structure. The basic formula of a tetrahedral sheet is $Si_2O_5^{2-}$, but increasing charge negativity, a critical determinant in the adsorptive capacity of clay minerals, can result from isomorphic substitution of Si^{4+} by trivalent metal cations such as Al^{3+} and Fe^{3+}. Octahedral sheets are comprised of two planes of OH^- groups, with octahedral sites occupied by cationic metals (typically Fe^{2+}, Mg^{2+}, Al^{3+} or Fe^{3+}) displaying six-fold octahedral coordination (i.e. six OH^- groups coordinated to one cation; radius ratio of cation/anion = 0.414-0.732). The valence of the octahedral cations differentiates octahedral sheets into two basic types to accommodate for charge balance; 1) trioctahedral sheets, in which divalent cations (e.g. Fe^{2+} or Mg^{2+}) occupy three out of three octahedral sites with the ideal formula $Mg_3(OH)_6$; and 2) dioctahedral sheets, in which trivalent cations (e.g. Al^{3+} or Fe^{3+}) occupy two out of three octahedral sites with the ideal formula $Al_2(OH)_6$ (Nesse, 2000).

The bonding observed between tetrahedral and octahedral sheets occurs via removal of an OH^- group from the octahedral sheet, which allows the apical oxygen atom in the tetrahedral sheet to form part of the adjacent octahedral sheet. On the basis of constituent sheet arrangement, clay minerals are predominantly characterized as exhibiting one of two layer structures (although mixed layer types do occur); 1:1 clay minerals, in which the layer structure consists of repeating tetrahedral-octahedral (TO) sheet subunits; and 2:1 clay minerals, in which the layer structure consists of repeating tetrahedral-octahedral-tetrahedral (TOT) sheet subunits (see Figure 1.2). Layer structure affects the interlayer spacing and swelling properties of a clay mineral, which influence the mineral's CEC, such that swelling clay minerals (e.g. smectites) exhibit higher CEC than non-swelling clay minerals (e.g. kaolinite); this corresponds to the abundant interlayer surface area available for cation exchange in swelling clay minerals (Maurice, 2009). The interlayer space between individual subunits may remain void, or can contain water molecules and/or interlayer cations which increase the attraction between the net negatively-charged sheets (Nesse, 2000). In nature, three basic types of clay minerals exist; kaolinite, smectites, and illites (also referred to as mica-like clay minerals, attributed to compositional and structural similarities of illites to muscovite) (Nesse, 2000, Babel & Kurniawan, 2003).1:1 clay minerals typically exhibit an interlayer spacing of ~7Å, are electrically neutral, are bonded via van der Waals interactions and H-bonding, and are relatively soft compared to 2:1 clay minerals, due to the weak nature of the electrostatic bonding observed in 1:1 clay minerals (Nesse, 2000). The dioctahedral clay mineral kaolinite ($Al_2Si_2O_5(OH)_4$) is an example of a 1:1 clay mineral, which features Al^{3+} cations in the octahedral sheets and SiO_4 tetrahedrons comprising the tetrahedral sheets (Bhattacharyya & Gupta, 2008a). 2:1 clay minerals exhibit a range in interlayer spacing between10-14Å. The tetrahedral-octahedral-tetrahedral layers of 2:1 phyllosilicates can be neutrally-charged (referred to simply as TOT, which feature weak electrostatic bonds, including van der Waals interactions and H-bonding), but clay minerals of 2:1 layer structure often demonstrate increased net layer charge negativity, resultant from isomorphic cation substitution into both tetrahedral and octahedral sites (Nesse, 2000). To balance the net negative charge characteristic of some 2:1 clay minerals, these minerals typically incorporate either: a) cationic metals (e.g. Ca^{2+}, Na^+, K^+, which are largely responsible for the CEC of clay minerals) into the interlayer, thus promoting the formation of both van der Waals interactions and ionic bonds in these TOT + c clay minerals; or b) an additional octahedral sheet in the interlayer, forming a

TOT + O clay mineral, in which the net negativity of the TOT subunit is compensated for by the net positive charge of the additional octahedral sheet (see Figure 1.2; Lothenbach et al., 1997, Sposito et al., 1999, Nesse, 2000, Carvalho et al., 2007, Bhattacharyya & Gupta, 2008a).

TOT + c clay minerals can be further characterized based on the magnitude of charge negativity of the TOT layers; low charge clay minerals (e.g. smectites) are those that feature a net negative charge between 0.2-0.6 per formula unit, and this charge is typically compensated for via the addition of Ca^{2+} and Na^{+} cations to the interlayer. High charge clay minerals (e.g. illites) are those with a net negative charge between 0.8-1.0 per formula unit, and this charge is typically compensated for via the addition of K^{+} cations to the interlayer. Importantly, low charge clay minerals swell upon exposure to water, while high charge clay minerals do not; this has significant implications on the CEC of a TOT + c clay mineral. Swelling in clay minerals reflects the ease with which water can enter the interlayer, such that low charge clay minerals, which contain relatively few interlayer cations and inherently weaker electrostatic bonding, swell, while water is unable to enter the interlayer of high charge clay minerals, due to the abundant interlayer cations and resultantly stronger electrostatic bonding (Nesse, 2000). The smectite clay mineral montmorillonite $((Si_{7.8}A_{l0.2})^{IV}(Al_{3.4}Mg_{0.6})^{VI}O_{20}(OH)_{4})$ is an example of a low charge 2:1 clay mineral (TOT + c), and features tetrahedral silica layers separated by an alumina octahedral layer (Bhattacharyya & Gupta, 2008a).

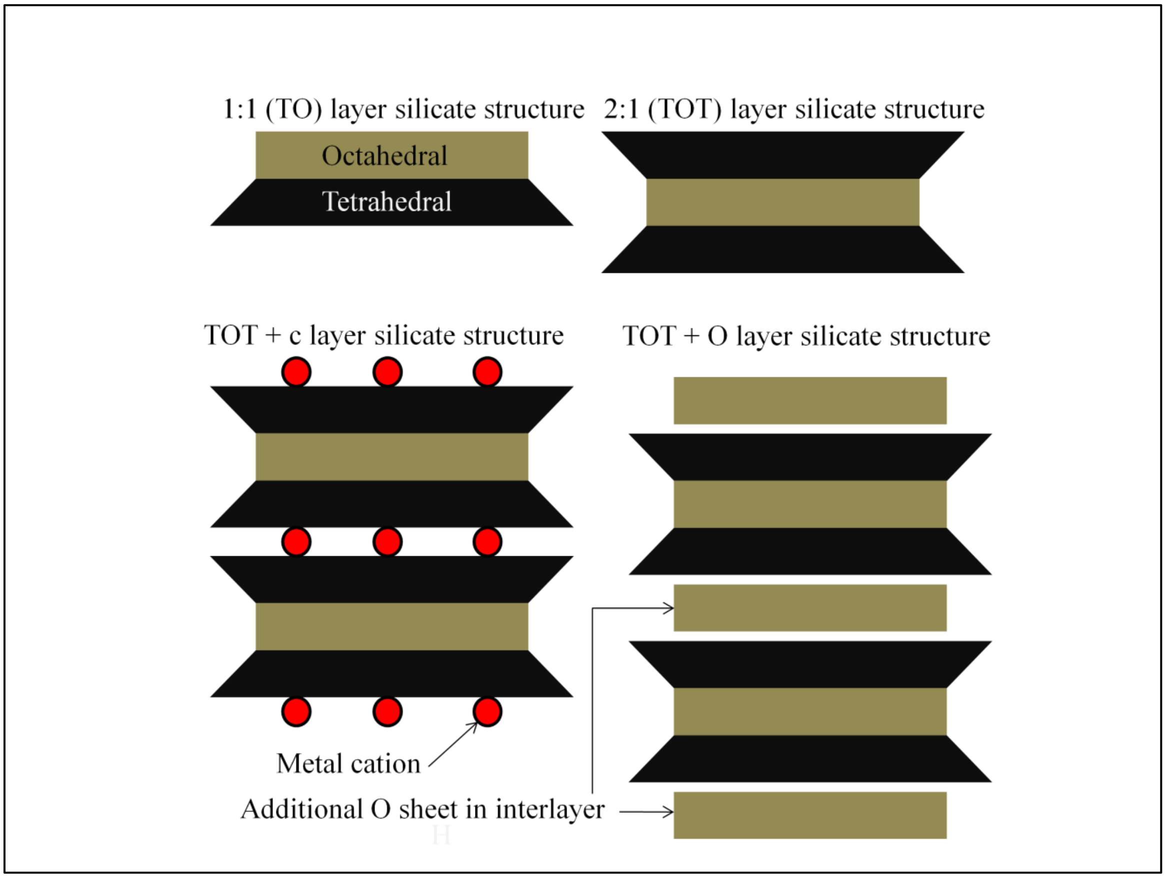

Fig. 1.2 – Schematic of clay mineral layer structures, including 1:1 clays comprised of repeating subunits of tetrahedral ($Si_2O_5^{2-}$)-octahedral ($Mg_3(OH)_6$, $Al_2(OH)_6$) sheets, and 2:1 clays comprised of repeating subunits of tetrahedral-octahedral-tetrahedral sheets. Mechanisms of layer charge balance in 2:1 clays include incorporation of cationic metals into the interlayer (TOT + c) and incorporation of an additional octahedral sheet into the interlayer (TOT + O). Modified from Nesse (2000).

1.3.2 Clay Mineral Metal Adsorption

Charge density of a mineral surface plays a key role in affecting the adsorption of metals. The charge density of a clay mineral particle (σ_P) is derived from the influence of intrinsic charge density (σ_{in}, which reflects both permanent structural charge, resulting from isomorphic substitution in the mineral lattice (σ_O), and pH-dependent proton surface charge density (σ_H)), along with charge density attributed to ions in association with the surface (i.e. inner-sphere, σ_{is}, and outer-sphere, σ_{os}, complexes); thus, $\sigma_P = \sigma_O + \sigma_H + \sigma_{is} + \sigma_{os}$ (Sposito, 1989, Lothenbach et al., 1997, Sposito et al., 1999, Carvalho et al., 2007, Bhattacharyya & Gupta, 2008a, Maurice, 2009). σ_O is largely balanced by the presence of interlayer cations (e.g. K^+, Na^+, Ca^{2+}) adsorbed around edges and between unit layers; interlayer cations, depending upon the swelling characteristics of the clay mineral, can thereafter be made available for cation exchange (Lothenbach et al., 1997, Bhattacharyya & Gupta, 2008a). The pH-dependent charge on clay mineral surfaces is observed at edge sites and results from protonation/ deprotonation of surface hydroxyl groups corresponding to pH fluctuation in the surrounding aqueous media (Lothenbach et al., 1997). Cationic metal uptake by clay minerals can involve complexation with surface hydroxyl groups, as well as cation exchange reactions (Stumm & Morgan, 1996). Clay mineral surface negativity facilitates the establishment of an electrical double layer on the clay mineral surface upon exposure of the mineral grains to aqueous solution containing dissolved ions (Sposito et al., 1999).

Cation exchange reactions involve the replacement of a readily exchangeable cationic species associated with a solid substrate by a different cationic species present in the surrounding aqueous solution, without inducing structural alteration to the substrate (Sposito, 1989, Bhattacharyya & Gupta, 2008b, Maurice, 2009). In the above definition, the term *readily exchangeable* refers strictly to fully solvated cations (i.e. those adsorbed via outer-sphere complexation or via incorporation into the diffuse-ion swarm); cations associated with the solid substrate via inner-sphere complexation are not readily exchangeable, and thus are not considered to influence the CEC of a soil or sediment. The term cation exchange capacity therefore refers to the maximum quantity of fully solvated cations per unit mass of soil/sediment (under conditions of specified pressure, temperature, solution composition, etc.). Micas and swelling clay minerals with negative structural charges typically exhibit high CEC, while non-swelling clay minerals typically feature substantially lower CEC. For instance, the swelling smectite clay minerals exhibit a CEC of 70-120cmols/kg, in comparison to non-swelling kaolinite, which exhibits a CEC of 1-15cmols/kg. This observed difference in CEC characterizing swelling versus non-swelling clay minerals reflects the ability of external surfaces as well as interlayer regions of swelling clay minerals to undergo cation exchange, while the interlayer region of non-swelling clay minerals remains inaccessible for cation exchange (Sposito, 1989, Maurice, 2009).

A number of physico-chemical factors affect adsorption of metals onto clay minerals including: 1) solution properties (e.g. pH, ionic strength, temperature, identity of electrolyte); 2) redox conditions; 3) saturation state of the aqueous media with respect to the adsorbent and the adsorbate; 4) speciation, concentration, and characteristics of metal adsorbate ions (e.g. charge to radius ratio, formation of oxyanions); 5) presence, concentration, and characteristics of ionic species and ligands in solution and on the adsorbent surface that may compete with cations; 6) presence and concentration of anionic species (e.g. sulphate, phosphate, carbonate) that may facilitate metal precipitation; 7) characteristics of adsorbent material (e.g. charge of clay mineral surface, density and nature of surface sites, micromorphology, presence of surface coatings and structural impurities); 8) abundance of adsorbent material; 9) type and stability of adsorption complex formed; and 10) physical and chemical modifications made to the adsorbent (Brown et al., 1999, Bradl, 2004, Adebowale et al., 2006, Potgieter et al., 2006, Kubilay et al., 2007, Bhattacharyya & Gupta, 2008a,b, Ijagbemi et al., 2009, Maurice, 2009).

Numerous studies have assessed abiotic cationic metal adsorption by natural and modified clays in the context of environmental remediation. The specific influence of pH on metal adsorption to clay minerals has been investigated by several authors (e.g. Gu & Evans, 2007, Kubilay et al., 2007, Li et al., 2009), generally showing greater cationic adsorption as pH increases. Divalent metal uptake by Ca-montmorillonite-rich bentonite clay was evaluated by Kubilay et al. (2007), and under low pH, gradual increase in adsorption with increasing pH was reported, followed by a sharp increase at pH > 7.0, which was attributed to exceedance of the pH_{PZC} (6.35) of the adsorbent. Similar conclusions were reached by Li et al. (2009), who examined Cu^{2+} adsorption onto Na-bentonite and showed that uptake increased gradually with pH below 5.0, rose sharply between pH 5.0-6.5, reaching a maximum rate of adsorption at this pH range. Ionic strength dependence was observed at pH < 6.5, which is indicative of a non-specific mechanism of adsorption (e.g. ion exchange with Na^+, outer-sphere complexation). Coinciding with the pH_{PZC} of the Na-bentonite (6.3+/-0.1), Cu^{2+} uptake continued at the maximum rate above pH 6.5; thus, upon exceeding pH_{PZC}, uptake is dominated by specific adsorption at deprotonated surface sites, inducing the formation of stable inner-sphere surface complexes with Cu species such as $Cu(OH)_2^0$, $Cu(OH)^+$ and $Cu_2(OH)_2^{2+}$ (Li et al., 2009). These studies show that cationic metal adsorption onto clay mineral substrates occurs more efficiently at higher pH (specifically, beyond pH_{PZC} of the adsorbent), and that pH conditions will determine the type and stability of adsorption complexes that form.

Ionic strength and pH were shown by Gu & Evans (2007) to significantly influence metal adsorption onto the clay mineral illite, a weathering product of micas. When evaluated in a 0.1M

$NaNO_3$ electrolytic solution, adsorption of Ni^{2+}, Zn^{2+}, and Cd^{2+} was gradual from pH 3.0-6.0, and increased rapidly from pH 6.0-7.0, reaching completion at pH 7.0. Adsorption of all metals in a 0.01M $NaNO_3$ electrolyte was shown to increase from ~20-40% to >70% in the pH range 3.0-5.0 and reach completion rapidly beyond pH 6.0. Finally, in a 0.001M $NaNO_3$ electrolyte solution, adsorption went to completion upon reaching pH 5.0. The observed ionic strength effects on metal adsorption reflect increased competition with Na^+ for adsorption sites at higher ionic strength. In addition, two distinct pH-dependent adsorption processes were found to dominate metal uptake onto illite under constant ionic strength; from pH 3.0-6.0, adsorption was highly influenced by ionic strength, which is indicative of a non-specific mechanism of adsorption, in this case involving cation exchange with Ca^{2+} and Mg^{2+} at frayed edges and the basal surface. At pH > 6.0, uptake occurred independently of ionic strength and proceeded to completion, supporting a dominance of inner-sphere complexation at edge sites. (Gu & Evans, 2007). This study illustrates the importance of ionic strength effects on the adsorption of metal cations to clay minerals in the context of competition for adsorption sites, and further supports the pH-dependant nature of adsorption.

Divalent metal adsorption to bentonite clay was evaluated as a function of temperature by Kubilay et al. (2007), who identified that adsorption occurred preferentially at low temperature (i.e. 20°C > 50°C > 75°C > 90°C). This result is indicative of an exothermal nature of adsorption, with preferential uptake of $Zn^{2+} > Cu^{2+} > Co^{2+}$ at pH 3.0, 5.0, and 7.0, and $Zn^{2+} > Co^{2+} > Cu^{2+}$ at pH 9.0 and 20°C. The observed change in metal affinity at alkaline pH results from preferential adsorption of more easily-hydrolysable metals, including Zn^{2+} and Co^{2+}, over less hydrolysable cations such as Cu^{2+} (Kubilay et al., 2007). The temperature-dependent nature of metal adsorption represents a potential limitation of clay adsorbent usage, supporting that clay minerals are best suited for use in low-temperature environmental systems.

Metal adsorption onto soil mineral constituents was examined by Sipos et al. (2009), who concluded that swelling clay minerals (e.g. smectites, vermiculite) demonstrate higher adsorption capacities in comparison with non-swelling clay minerals (e.g. illite) and micas, reflecting the higher specific surface area and higher CEC of swelling clay minerals. To assess the metal scavenging abilities of a naturally-analogous clay mineral assemblage, multi-mineral phases were created and evaluated, including chlorite/vermiculite, illite/vermiculite/smectite, and illite/smectite. Illite/vermiculite/smectite and illite/smectite phases yielded similar adsorption capacities to illite, while the chlorite/vermiculite phase was found to adsorb the largest quantity of Cu^{2+} and similar quantities of Pb^{2+} and Zn^{2+} to the swelling clay minerals. Adsorption efficiency was found to vary depending upon the buffering capacity of the substrate, such that clays with higher buffering capacity (i.e. larger carbonate fraction that can provide acid neutralizing capacity, ANC) immobilized greater quantities of metals. Enhanced adsorption onto chlorite/vermiculite was attributed to the carbonate content of this phase, which resulted in an equilibrium pH of the batch adsorption solution of 5.82; in comparison, other equilibrium solutions ranged in pH between 4.0-4.5. Cu^{2+} adsorption occurs inner-spherically, which requires higher solution pH to facilitate deprotonation of surface sites rendering them available for specific adsorption. Unlike Cu^{2+} and Pb^{2+}, which preferentially associate with the surface as inner-sphere complexes (assuming $pH > pH_{PZC}$), Zn^{2+} is primarily adsorbed non-specifically to clay mineral surfaces. (Sipos et al., 2009).

Usman et al. (2005) examined the impact of soil amendment with Na- and Ca-bentonite clays on the mobility of loosely-bound metals in soils contaminated with sewage sludge, and observed a substantial decrease in the water-extractable and exchangeable metal fractions of amended versus non-amended soils. The adsorptive properties of the bentonite additives were attributed to the abundance of montmorillonite (62-70%) comprising the samples, and this study suggests that bentonite augmentation of metal-polluted soils and sediments can serve as a viable *in situ* remediation option. (Usman et al., 2005). Kugler et al. (2002) examined the retention of metal cations by a kaolinite-rich landfill liner to assess the penetration depth of metallic contaminants after ten years of exposure to landfill waste and leachate. The authors compared the extractability of metals from uncontaminated liner samples subjected to batch adsorption experiments in the laboratory with the extractability of metals retained *in situ* via exposure to landfill leachate. Batch adsorption experiments showed that metal uptake followed the affinity series $Cd^{2+} < Zn^{2+} < Cu^{2+} < Pb^{2+}$, but ~20% of the metal load retained by the liner material during batch adsorption experiments was readily remobilized by deionized water, following the mobility sequence $Pb^{2+} < Cu^{2+} < Zn^{2+} < Cd^{2+}$. Significant remobilization was also observed in the exchangeable fraction, according to the mobility sequence $Zn^{2+} < Cu^{2+} < Pb^{2+} < Cd^{2+}$. Remobilization of metals in these fractions indicates the prevalence of weaker, nonspecific adsorption controlling metal uptake under the experimental conditions, while sequential extraction of the liner material contaminated *in situ* with landfill leachate indicated the prevalence of comparatively stronger adsorption mechanisms. Observed stability differences in adsorption complexes reflect the degree of metal loading experienced by samples subjected to adsorption experiments compared to those contaminated *in situ*. Leachate samples taken from the landfill were less metal-laden than the experimental solutions, indicating that exposure to elevated metal concentrations favoured the prevalence of weaker surface complexation. This study concluded that the kaolinite-based liner successfully retained metals within the top two centimetres. (Kugler et al., 2002). The results of Kugler et al. (2002), Usman et al. (2005), and Sipos et al., (2009) collectively underscore the potential of natural (or naturally analogous) clay mineral assemblages as cationic metal adsorbents.

The influence of Al-modification of the smectite montmorillonite on metal uptake was assessed by Lothenbach et al. (1997), who compared adsorption onto unmodified montmorillonite and two forms of modified montmorillonite (Al-montmorillonite [hydroxyl-aluminum montmorillonite with $Al(OH)_3$ coatings and intercalations]; and Al_{13}-montmorillonite [montmorillonite pillared with the polynuclear aluminum complex Al_{13}]). Changes in the surface properties were observed with Al-modification, including increased surface area ($20m^2/g$ for unmodified montmorillonite, $60m^2/g$ for Al-montmorillonite, $220m^2/g$ for Al_{13}-montmorillonite) and decreased CEC (86cmol/kg for the unmodified clay mineral, 14cmol/kg for Al-montmorillonite, 4cmol/kg for Al_{13}-montmorillonite). Under low pH (~4-5), unmodified montmorillonite adsorbed more efficiently than Al-montmorillonite or Al_{13}-montmorillonite, with considerable remobilization of adsorbed cations occurring upon exposure to Ba^{2+}, indicating the electrostatic (i.e. nonspecific) nature of adsorption via cation exchange onto the unmodified montmorillonite. Conversely, Al-modification accommodated for enhanced metal uptake under alkaline pH; Ni^{2+}, Cu^{2+}, and Zn^{2+} were negligibly remobilized, supporting that the adsorption of these metals occurred via inner-sphere complexation onto the Al-modified clay minerals. Observed differences in predominant adsorption mechanisms demonstrated by Al-modified montmorillonites and the pure clay mineral led to the conclusion that Al-modification significantly reduces the number of low-affinity cation exchange sites on the substrate by replacing them with high-affinity, pH-dependent aluminum hydroxyl functional groups; this explains the preferential uptake demonstrated by unmodified montmorillonite under lower pH and the more efficient adsorption of metals demonstrated by Al-modified clay minerals under more alkaline pH. In the context of remediation, this supports that Al-modified montmorillonite can encourage the specific adsorption of Ni^{2+}, Cu^{2+}, and Zn^{2+}, rendering these cations relatively immobile and unavailable for cation exchange with naturally occurring divalent metals, such as Ca^{2+} and Mg^{2+}. The authors recommend the use of Al-modified montmorillonite in remediation efforts of systems at pH 6.0 and greater, due to the strong inner-spherical complexation observed, while the use of unmodified montmorillonite is recommended at pH < 5 due to the greater CEC demonstrated by the pure clay mineral. (Lothenbach et al., 1997, and references therein). Thus, modification of pure clay mineral phases prior to application as adsorbents may act to enhance the efficiency of metal uptake in particular environmental systems, while potentially retarding metal uptake in others.

In summary, several important physico-chemical factors have been shown to control cationic metal adsorption onto clay mineral substrates. A strong positive correlation is observed between increasing solution pH and adsorption efficiency; adsorption of cationic metals is enhanced under increasingly alkaline conditions until reaching a metal-specific pH, above which the precipitation of metal hydroxides dominates over adsorption (Potgieter et al., 2006, Abollino et al., 2008, Bhattacharyya & Gupta, 2008b), and typically decreases under low-pH conditions reflecting the intensified competition between cationic metals and protons for surface sites (Kubilay et al., 2007, Abollino et al., 2008, Bhattacharyya & Gupta, 2008b, Ijagbemi et al., 2009).

Metal adsorption has also been identified to vary as a function of temperature, with optimal adsorption of most metal species occurring at lower temperatures (~20°C) and desorption of cationic species occurring at elevated temperatures (Kubilay et al., 2007, Bhattacharyya & Gupta, 2008b). This relationship reflects the dominantly exothermic nature of the adsorption process (Kubilay et al., 2007, Bhattacharyya & Gupta, 2008b). Element-specific exceptions, such as Cr(VI) and Sr(II) are detailed by Babel & Kurniawan (2003).

Ionic strength dependence of metal adsorption onto illite was demonstrated by Gu & Evans (2007) to be particularly prevalent below pH 6.0; above pH 6.0, inner-sphere complexation dominated, and thus ionic strength effects became negligible. The influence of ligand presence on metal adsorption to clay minerals is found to vary depending upon the characteristics of the clay mineral adsorbent, the cationic metal species of interest, and the ligand under consideration. Adsorption is enhanced by the presence of ligands which encourage the formation of negatively charged complexes (i.e. organics) that can thereafter interact with surface sites of positive charge; these positively charged surface sites would be otherwise unavailable for metal adsorption. Adsorption is also enhanced via formation of outer-sphere complexes, which result from interactions between metal cations and clay mineral surface-ligand complexes of negative charge. Alternatively, ligands can bind metals as soluble complexes that do not then adsorb to clay minerals, and thus effectively decrease the observed adsorption efficiency. Similarly, ligands can also compete with metal cations for surface sites, thereby reducing the number of accessible surface sites for metal adsorption (Abollino et al., 2008). Thus, the influence of ligands on adsorption efficiency greatly depends upon the characteristics of the cationic metal species, the clay mineral surface, and the ligand itself.

The quantity of substrate affects metal uptake such that adsorption is enhanced when adsorbent concentrations increase; however, when evaluated in terms of *efficiency* of the adsorbent (i.e. mass of metal adsorbed per unit mass of adsorbent), an excess of adsorbent promotes a decrease in adsorption efficiency (Chen et al., 2007, Ijagbemi et al., 2009). This reflects the larger number of surface sites available when the adsorbent is present in excess, and the preferential adsorption of cations onto more energetically favourable sites, which leaves a greater number of adsorption sites (per unit mass adsorbent) unfilled (Chen et al., 2007, Ijagbemi et al., 2009). Adsorbate concentration influences metal adsorption such that uptake from dilute solution occurs independently of concentration due to the large proportion of surface sites available, corresponding to a lack of competition for sites (Bhattacharyya & Gupta, 2008b). Increased adsorbate concentration, however, typically results in a decrease in adsorption efficiency, as competition for available surface sites increases (Bhattacharyya & Gupta, 2008b). Metal sequestration from more highly concentrated solutions involves the adsorption of cations onto energetically less favourable surface sites, thus further reducing metal removal efficiency (Kubilay et al., 2007).

Finally, various chemical processes have been identified to enhance the adsorptive properties of clay minerals. Acid activation involves exposure to strong inorganic acids at high temperature, which causes crystal edges to open, induces removal of carbonate fractions, facilitates exchange of clay mineral surface cations with protons, replaces cations (e.g. Mg^{2+}, Al^{3+}) from octahedral sheets, decreases the degree of crystallinity, increases porosity and pore size, and results in increased surface area corresponding to removal of impurities from the clay matrix (e.g. Ravichandran & Sivasankar, 1997, Suraj et al., 1998, Chen et al., 2007, Bhattacharyya & Gupta, 2008a, Eloussaief et al., 2009, Kooli, 2009, Korichi et al., 2009, Steudel et al., 2009a,b). Activation of kaolinite and palygorskite induced the formation of additional aluminol (>Al-OH) and silanol (>Si-OH) groups, which then served as adsorption sites (Suraj et al., 1998, Chen et al., 2007). Numerous protocols for acid activation have been described in the literature, commonly employing strong inorganic acids such as hydrochloric (HCl) or sulphuric (H_2SO_4) acids in varying concentration (e.g. Suraj et al., 1998, Chen et al., 2007, Kooli, 2009). Pillaring is a second process documented to improve the adsorption capacity of clay minerals by actively increasing the layer spacing between sheets through the addition of ceramic oxide, metal oxide, or other particles to the interlayer space (e.g. Bhattacharyya & Gupta, 2008a). Increasing the interlayer space accommodates for ease of migration of ions from the surrounding aqueous media into the interlayer space of the clay mineral adsorbent, thereby enhancing the accessibility of active sites to incoming adsorbate ions. Several authors (e.g. Lothenbach et al., 1997, Suraj et al., 1998, Ikhsan et al., 1999, Papini et al., 2001, Bostick et al., 2002, Cooper et al., 2002, Babel & Kurniawan, 2003, Chorover et al., 2003, Chantawong et al., 2003, Yavuz et al., 2003, Usman et al., 2005, Adebowale et al., 2006, Dal Bosco et al., 2006, Potgieter et al., 2006, Abollino et al., 2007, Carvalho et al., 2007, Gu & Evans, 2007, Kubilay et al., 2007, Veli & Alyüz, 2007, Abollino et al., 2008, Bedoui et al., 2008, Bhattacharyya & Gupta, 2008a,b, Chaari et al., 2008, Ribeiro et al., 2008, Akar et al., 2009, Ijagbemi et al., 2009, Li et al., 2009, Sipos et al., 2009) have demonstrated the use of clay minerals as adsorbents for metal cations. Although beyond the scope of this review, current evidence also indicates the ability of clay minerals to adsorb dissolved organics as well as numerous non-ionic and anionic species from aqueous solution (Cervini-Silva et al., 2000, Tietjen et al., 2005, Abollino et al., 2008, Bhattacharyya & Gupta, 2008a).

The current literature is dominated by studies such as those discussed above which examine strictly abiotically-driven metal adsorption onto clay mineral substrates. As one of the few studies to consider biologically-influenced adsorption of metal cations coupled with a clay mineral substrate, the work of Akar et al. (2009) assessed the adsorption of Cu^{2+} onto montmorillonite-immobilized cells of the fungi *Trametes versicolor*. Batch adsorption experiments showed that Cu^{2+} uptake was minimal from pH 1.0-3.0, increased from pH 3.0-5.0, and reached maximum adsorption at pH 5.0; above pH 6.0, precipitation of Cu^{2+}-hydroxide phases dominated. The increase in adsorption from pH 3.0-5.0 was attributed to the deprotonation of surface sites on the montmorillonite/*Trametes versicolor* composite and subsequent inner-sphere complexation. (Akar et al., 2009). Substantive literature, as discussed subsequently, now exists evidencing the importance of microbes as both geochemically reactive surfaces involved in metal sorption and as agents influencing metal sorption through metabolic processes. The relative lack of microbially-clay mineral-coupled metal adsorption studies in the current literature suggests that room for growth in this field exists, and significant discoveries will derive from research assessing the direct influence of microorganisms on the adsorption of metals to clay mineral substrates which can occur via a number of processes as discussed subsequently.

1.4 Microbe-Mineral Interactions

1.4.1 Introduction to Microbial-Mineral Interactions

Research efforts have identified numerous interactions that occur between microorganisms and mineral substrates, and these interactions have been well documented in the literature. Interactions of particular significance to this review are those with the ability to affect the metal scavenging characteristics of clay minerals, and include sorption of siderophores to clay minerals and the influence of siderophores on metal sorption by clay minerals (e.g. Neubauer & Furrer, 1999, Neubauer et al., 2000, Neubauer et al., 2002, Siebner-Freibach et al., 2004, Haack et al., 2008), microbial weathering of mineral phases (e.g. Bennett et al., 1996, Liermann et al., 2000, Bennett et al., 2001, Edwards & Rutenberg, 2001, Maurice et al., 2001b, Dong et al., 2003, Buss et al., 2007, Mailloux et al., 2009, Uroz et al., 2009), microbial alteration of mineral phases (e.g. Kostka et al., 1999a, Kostka et al., 2002, Kim et al., 2004, O'Reilly et al., 2006, Stucki & Kostka, 2006, Jaisi et al., 2007, Kashefi et al., 2008, Müller, 2009, Mulligan et al., 2009), microbial influences on clay flocculation (e.g. Kim et al., 2005, Jaisi et al., 2007), and preferential colonization of nutrient-bearing mineral phases (e.g. Roberts, 2004, Rogers & Bennett, 2004). Indeed, bacterial growth and metabolic activity has thus been shown to display sensitivity towards the presence and abundance of certain clay minerals (e.g. Maurice et al., 2001a, Courvoisier & Dukan, 2009). Microorganisms mediate numerous geochemical processes and exhibit a significant influence on the geochemical cycling of metals and other elements including C, N, P, and S (Edwards & Rutenberg, 2001, Lloyd & Lovley, 2001, Gadd, 2004). Among the geochemical processes governed by the activity of microorganisms are mineral formation (e.g. Hama et al., 2001, Konhauser et al., 2002, Jackson et al., 2011) and dissolution (e.g. Edwards & Rutenberg, 2001). As evidence increases (e.g. Bennett et al., 1996, Rogers & Bennett, 2004, Mailloux et al., 2009), it is clear that microbially-induced mineral dissolution is a mechanism by which microbes can obtain important nutrients for use in life processes; in doing so, clay minerals may be generated, altered, or dissolved, ultimately affecting overall clay mineral sorbent characteristics. Mineral weathering thus assumes an important role in controlling the bioavailability of nutrients and toxic elements in environmental systems. The acquisition of nutrients facilitated by microbial mineral weathering can induce the ancillary release of toxic elements, including metals and metalloids (Mailloux et al., 2009).

1.4.2 Microbial Siderophore Influence on Clay Minerals

Siderophores are low-molecular weight organic chelating molecules with a high affinity for iron, which possess as many as six N or O electron donor atoms, and are released by microorganisms under conditions of iron deficiency to promote mobilization of Fe from soil minerals (Hernlem et al., 1996, Neubauer & Furrer, 1999, Liermann et al., 2000, Neubauer et al., 2000, Neubauer et al., 2002, Siebner-Freibach et al., 2004, Buss et al., 2007, Uroz et al., 2009). Additionally, siderophores have been shown to chelate and form stable complexes with a number of divalent metal cations, although such complexes are typically less stable than siderophore-Fe complexes (e.g. Hernlem et al., 1996, Neubauer et al., 2000 and references therein). Consequently, organic ligand complexation of metals is regarded as an important control affecting metal solubility in environmental systems (Neubauer & Furrer, 1999). Commonly, bacterial siderophores are catecholates, while fungal siderophores are hydroxamates (Neubauer et al., 2000). Within the scope of this review, siderophores have been shown to not only interact with divalent metal cations, thereby influencing metal sorption to clay minerals, but have also been shown to interact with clay minerals directly through processes such as adsorption and siderophore-induced structural Fe^{3+} reduction (Neubauer et al., 2000, Neubauer et al., 2002, Siebner-Freibach et al., 2004, Haack et al., 2008).

Neubauer et al. (2000) investigated metal sorption by the clay minerals montmorillonite and kaolinite with and without the presence of desferrioxamine B (DFOB), a linear, protonated, hydroxamate siderophore (Siebner-Freibach et al., 2004). Fe chelation by DFOB occurs via bonding between Fe and the carbonyl oxygens as well as the deprotonated hydroxyls of the hydroxamate groups, which results in the formation of a highly stable complex (Neubauer & Furrer, 1999, Siebner-Freibach et al., 2004). By augmenting montmorillonite and kaolinite suspensions with DFOB, the influence of DFOB on metal sorption to the clay minerals was studied and produced significantly different results for the two clay minerals investigated; the authors propose that this difference corresponds to the significant variation in permanent negative surface charge characterizing the two clay mineral substrates. Metal immobilization by montmorillonite in the presence of DFOB was shown to demonstrate both metal- and pH-dependence; Cu^{2+} immobilization, for instance, was enhanced across the pH range 4-10, while Cd^{2+} and Zn^{2+} sorption was enhanced until reaching sorption maxima at pH 8 and 7, respectively. DFOB-enhanced sorption by montmorillonite is explained to occur as a result of electrostatic interactions between the positively charged metal-DFOB complexes, which allow the complexes to be sorbed via cation exchange at low pH and via specific binding at higher pH. Decreased sorption of Cd^{2+} and Zn^{2+} above pH 9 and 8, respectively, corresponds to the formation of neutrally-charged metal-DFOB complexes which dominate in this pH range, while maxima at pH 8 and 7 correspond to the prevalence of positively-charged metal-DFOB complexes in this range. Contrarily, metal sorption to kaolinite was negatively influenced by the presence of DFOB across the pH range 4-10, with the exception of some limited sorption of Cd^{2+} and Zn^{2+} occurring above pH 6. Metal-DFOB complex adsorption onto kaolinite is explained by the authors to correspond to adsorption onto variably charged sites. In general, the observed difference in metal-DFOB sorption to the two clay minerals was attributed to the negativity of the mineral surface, inherently explained by the degree of isomorphic substitution in the mineral lattice. (Neubauer et al., 2000). Results of the work of Neubauer et al. (2000) are largely supported by those of Neubauer & Furrer (1999), who found that DFOB augmentation to montmorillonite suspensions enhanced the adsorption of Zn^{2+} and Cd^{2+} as a result of electrostatic interactions between the negatively charged clay mineral surface and the positively charged DFOB-metal complexes. Cd^{2+} and Zn^{2+} adsorption reached maximum values at pH 8 and pH 7 in the presence of DFOB, in agreement with the values obtained by Neubauer et al. (2000).

Metal sorption to montmorillonite and kaolinite was studied by Neubauer et al. (2002) in the presence of DFOB + Fe^{3+} to examine the influence of Fe^{3+} on the sorption characteristics of divalent metals in solution with DFOB. Results showed that the influence of DFOB on Cd^{2+} and Cu^{2+} sorption to montmorillonite (as reported in Neubauer et al., 2000) was diminished and became negligible with the excess addition of dissolved Fe^{3+}. Cu^{2+} sorption to kaolinite in the presence of DFOB alone was hindered as shown by Neubauer et al. (2000); with dissolved Fe^{3+} in excess of DFOB concentrations, solubility of Cu^{2+} showed similar trends to those observed in the absence of DFOB altogether, although the sorption edge occurred at a lower pH. These results support the preferential complexation of $Fe^{3+}_{(aq)}$ with DFOB, which effectively renders the siderophore unavailable for complexation with divalent metals, thereby negating the effects of the siderophore on metal adsorption to kaolinite and montmorillonite.

Neubauer et al. (2000) also investigated the sorption of DFOB onto montmorillonite and kaolinite, and found that DFOB demonstrates a strong affinity for montmorillonite and a weak affinity for kaolinite. The same affinity trend was reported by Siebner-Freibach et al. (2004), who investigated the sorption of DFOB and the stable Fe-complexed variation of this siderophore, ferrioxamine B (FOB), onto montmorillonite and kaolinite. In the study of Neubauer et al. (2000), DFOB was sorbed to a concentration of roughly 200μmol/g montmorillonite under acidic conditions and showed decreased affinity above pH 7, corresponding to a decrease in the positive charge of DFOB above this pH. DFOB sorption onto kaolinite was observed to positively correlate with pH, but in comparison to montmorillonite, uptake onto kaolinite was minimal (i.e. 1-5% of the initial 298μM solution concentration was sorbed) (Neubauer et al., 2000). Siebner-Freibach et al. (2004) observed similarities between the adsorption isotherms for DFOB and FOB onto Na- and Ca-montmorillonite, which was interpreted to imply the predominance of a similar adsorption mechanism onto both variations (i.e. cation exchange). The adsorption of FOB occurred to a lesser extent than DFOB on both Na- and Ca-montmorillonite, which was explained by steric hindrance, resultant from the wider configuration of the FOB complex, and/or exposure of hydroxyl group and carbonyl oxygen polar sites in the linear DFOB molecule; these functional groups encourage adsorption via the formation of H-bonds, but are unavailable for adsorption of FOB, as they are bound to iron. Adsorption onto kaolinite was sufficiently lower than to montmorillonite, and was not found to reach the CEC of kaolinite; this is attributed primarily to steric hindrance and inability to interact with charged sites in the clay mineral lattice.

1.4.3 Mechanisms of Microbial Mineral Weathering

Mineral weathering is greatly enhanced by the activity of microorganisms via the production and excretion of biogenic substances and through microbially-mediated redox reactions (Uroz et al., 2009) that can be both passive (non-targeted mineral weathering) and active (targeted mineral weathering to acquire limiting nutrients). Biogenic substances including organic acids, enzymes, alcohols, extracellular polymeric substances (EPS), and chelating molecules are excreted by microorganisms and are thereafter observed to influence the dissolution of mineral phases by encouraging surface complexation, solution complex formation, influencing solubility through localized pH fluctuations, enhancing ligand-promoted dissolution, or catalysis of redox reactions (Buss et al., 2007, Uroz et al., 2009). The occurrence and extent of microbial mineral weathering processes is of interest to numerous issues, such as nuclear waste repository stability (e.g. Perdrial et al., 2009), and in the context of this review, the fate and stability of sorbed metals.

Microbial siderophores (see section 1.4.2) are found to play important roles in the dissolution of Fe-bearing mineral phases (Liermann et al., 2000, Buss et al., 2007, Haack et al., 2008, Uroz et al., 2009), and colonizing communities of microbes have been shown by Rogers & Bennett (2004) to encourage silicate matrix dissolution through the production of siderophores that complex with Si^{4+} and Al^{3+}. Their study showed that Fe^{3+} reduction and Si^{4+} mobilization in the presence of dissimilatory iron reducing bacteria (DIRB) were intimately connected; the simultaneous release of Si^{4+} and Fe^{2+} identified that DIRB can enhance silicate mineral weathering. Siderophore-promoted dissolution in the presence of *Bacillus sp.* resulted in the formation of localized clusters of etch pits, supporting that siderophore release can cause alterations in mineral surface topography (Buss et al., 2007). Rogers & Bennett (2004) state that etch pit formation is found to occur on mineral surfaces in direct contact with attached microbes, and postulate that the extent of surface weathering varies in direct proportion to the degree of microbial colonization. Imperfections in surface micro-topography caused by siderophore-induced etch pit formation is explained by Edwards & Rutenberg (2001) to encourage subsequent microbial adhesion, as microbes preferentially exploit and adhere to surface imperfections on mineral grains. Further, micro-topographical variations can alter the binding strength of a bacterium to a mineral surface, with energy of adhesion between microbe and substrate dependent upon surface geometry, bacterial morphology, and interaction potential. Buss et al. (2007) speculate that the localization of etch pits may be enhanced in the presence of EPS, which prevents the migration of siderophores away from the mineral surface and causes localized dissolution corresponding to elevated siderophore concentrations at the mineral surface (Buss et al., 2007). Additionally, EPS encourages localized dissolution by acting as a sink for ions released through substrate dissolution, but cannot alone cause the dissolution of hornblende glass; this suggests that ligand, and in particular, siderophore presence is conducive to the dissolution of Fe-bearing mineral phases, with EPS acting to enhance siderophore-promoted mineral dissolution (Buss et al., 2007).

Haack et al. (2008) examined the siderophore-induced dissolution of montmorillonite as well as sorption characteristics of DFOB, desferrioxamine D (DFOD), and acetohydroxamic acid (aHA) onto montmorillonite. Both DFOB and DFOD demonstrated high affinity for montmorillonite, while aHA sorption to the clay mineral was minimal. Mechanisms of organic absorption to smectites include interactions between interlayer cations or waters of hydration bound to interlayer cations with polar function groups on the organic molecule, as well as cation exchange; cation exchange is the proposed mechanism by which rapid uptake of DFOB and DFOD occur, while site-specific interactions occur subsequently. The authors additionally propose the occurrence of siderophore-induced cooperative mechanisms which influence DFOD and DFOB absorption onto the clay mineral; such mechanisms are thought to involve the stabilization and encouragement of absorption by neighbouring absorbed molecules, for instance via ligand-ligand interactions, or by ligand-induced changes in the clay mineral structure resultant from earlier ligand absorption. Siderophore influence on structural Fe^{3+} content of the clay mineral was dependent upon the siderophore involved and correlated with the absorption of the ligand to the clay mineral. Na-montmorillonite dissolution at pH 5.5 in the presence of DFOB and DFOD (which both absorbed appreciably to the clay mineral) was evidenced by increased dissolved Fe^{3+} and Al^{3+} concentrations. Minor amounts of Fe^{3+} and Al^{3+} in the presence of aHA, which did not absorb to a significant extent, indicated minimal dissolution of the smectite; similarly, no evidence of smectite dissolution was found in the absence of siderophores. Observed differences in siderophore-induced smectite dissolution were attributed to the low binding constants for metal-aHA complexes and the structure of aHA; contrarily, DFOD and DFOB demonstrate strong binding constants for Fe^{3+} and Al^{3+}. Further, the authors suggest that DFOD and DFOB may act to enhance smectite dissolution by encouraging proton-promoted dissolution or by promoting ligand-induced dissolution at smectite surfaces.

Mineral weathering can also be enhanced through microbially-mediated redox reactions, which have the potential to mobilize or immobilize metals, depending upon the metal species involved (Gadd, 2004). Many bacteria facilitate dissimilatory reduction reactions (e.g. *Shewanella sp.*, *Geobacter sp.*, Fe reduction) as well as dissimilatory oxidation reactions (e.g. *Acidothiobacillus sp.*, Fe and S oxidation) as catabolic mechanisms for acquiring energy for cellular processes (Liermann et al., 2007). For review on dissimilatory metal reduction, see Lovley (1993). Such metabolic processes are particularly influential in mediating the cycling of metals between abiotic and biotic components of environmental systems, with mineral weathering occurring predominantly through cellular respiration processes which utilize an alternative terminal electron acceptor to oxygen (e.g. SO_4^{2-}, NO_3^-, Fe^{3+}) (Liermann et al., 2007, Uroz et al., 2009). The movement of electrons to or from a solid mineral substrate requires unique cellular mechanisms, and to date, three mechanisms of electron transfer have been proposed and supported by current literature (citing Fe-oxide mineral phases as an example) (Liermann et al., 2007). These mechanisms include the use of low molecular weight electron shuttles; the production of chelating molecules, such as siderophores; and direct cell-mineral contact, which employs electron transfer between the mineral surface and the bacterial outer membrane (Liermann et al., 2007).

1.4.4 Preferential Colonization and Weathering of Mineral Phases

Preferential colonization specifically of nutrient- and micronutrient-bearing mineral and glass phases has been observed by several authors (e.g. Bennett et al., 1996, Rogers & Bennett, 2004). Rogers & Bennett (2004) suggest that microorganisms can derive a broad range of macro- and trace-nutrients, including Fe, K, Mn, Ni, and V, through microbially-induced weathering of silicate minerals and glasses. The observed microbial colonization of quartz, which features no apparent nutritional value, is attributed by Bennett et al. (1996) to the presence of trace micronutrients (e.g. Co, Cu, Fe^{3+}, Mo, Ni, and Zn) incorporated as inclusions and substitutions within the quartz crystal lattice. The extent of quartz colonization in the experiments of Bennett et al. (1996), however, was relatively low when compared with the colonization of K-bearing feldspars, which attracted an extensive microbial community of variable density and morphology. Further, K-rich feldspars (i.e. anorthoclase and microcline) were significantly more highly colonized than the Na-rich end member (i.e. albite), reflecting the K-deficient conditions existing within the experimental groundwater media. K- and Na-bearing anorthoclase was highly colonized, while Na-bearing albite remained uncolonized in their experiments, suggesting that mineral surface colonization by microbes is dependent upon the composite conditions of mineral composition, solution geochemistry, and nutrient requirements of the colonizing microorganism(s) (Bennett et al., 1996). Rogers & Bennett (2004) showed similar results, identifying that Fe- and P-bearing silicate glasses were preferentially colonized and weathered over glasses lacking these limiting nutrients.

The attachment of microorganisms to mineral surfaces also forms micro-environments which are chemically distinct from surrounding aqueous media, and may act as protection for the microorganisms from various environmental hazards (e.g. Uroz et al., 2009). Quartz and feldspar weathering rates observed in the colonization experiments of Rogers & Bennett (2004) were greatly enhanced when compared to abiotic controls, and concluded to result from elevated organic acid concentrations at the micro-environmental scale (Bennett et al., 1996, Rogers & Bennett, 2004). The micro-environment surrounding bacterial cells is chemically complex and reactive as a consequence of the microbial ability to influence dissolution and precipitation of mineral phases (Roberts, 2004). Silicate weathering is greatly enhanced by the activity of colonizing microbes, as evidenced by the development of etch pits and in some cases, the formation of secondary alteration products, including clay minerals (Bennett et al., 1996).

Roberts (2004) provides one of the few studies to date that investigates the importance of different factors influencing microbial colonization of mineral surfaces. This study suggests that, while coulombic attraction will dominate surface colonization between negatively charged microbial cells and positively charged mineral phases, the colonization of negatively-charged surfaces is dependent upon the composition of the mineral/glass phase in question. Positively charged mineral surfaces, including Fe- and Al-oxides are colonized through passive coulombic/electrostatic attraction; however, neutral or negatively charged mineral surfaces, including silicates, are less favourable electrostatically to the negatively charged microbial cell, and thus, the composition of the silicate phase (and therefore ecological desirability) dictates the extent of microbial colonization. Further, this study demonstrates that microorganisms will preferentially colonize mineral surfaces exhibiting nutritional benefit, while leaving surfaces containing toxic elements uncolonized. Experimental assessment indicated that microorganisms only colonized mineral phases containing minor quantities of Al, a metal known to demonstrate inhibitory effects upon microbial growth. However, this colonization of Al-bearing minerals was only observed to occur when the phase also contained nutrient elements beneficial to the microorganism, such as Fe. While solution pH and ionic strength were also observed to influence colonization, the most important factors controlling the microbial colonization of a mineral surface were determined to be mineral composition and surface charge. Extensive colonization of Fe-oxyhydroxide-coated quartz and plagioclase mineral grains was observed, while uncoated quartz grains exhibited only moderate colonization and uncoated plagioclase grains remained uncolonized. The attachment of microorganisms to mineral surfaces, subsequent colony growth, and production of EPS or other organic molecules can strongly influence the surface reactivity and surface charge of the mineral, and may further influence porosity and permeability characteristics of the colonized mineral substrate. (Roberts, 2004).

Results of Roberts (2004) underscore the need to consider microbial mineral weathering in clay-metal interactions. Kaolinite ($Al_2Si_2O_5(OH)_4$) is an abundant clay mineral, and is commonly found to contain trace Fe concentrations in the form of accessory minerals, structural substitutions, and surface precipitates (Maurice et al., 2001b). The trace concentrations of Fe commonly observed in kaolinite make this mineral a valuable source of Fe for bacteria, and in many natural systems, kaolinite dissolution is consequently dominated by microbial activity (Maurice et al., 2001b). In this study, enhanced microbial growth of the aerobic heterotroph *Pseudomonas mendocina* was observed in the presence of Fe-bearing kaolinite in comparison to control samples, which contained Fe-extracted kaolinite and were characterized by a relative decline in microbial population. Growth in the presence of Fe-bearing kaolinite supports the ability of *Pseudomonas mendocina* to obtain Fe from the mineral substrate by promoting kaolinite dissolution, which was further evidenced by elevated Al and Si solution concentrations relative to initial concentrations and those observed in control samples. These findings promoted Maurice et al. (2001b) to conclude that aerobic bacteria can assume important roles in the dissolution of nutrient-bearing clay minerals, while identifying that weathering of aluminosilicates is related to their potential as Fe sources for microorganisms in aerobic environments.

1.4.5 Microbially-Induced Structural Fe^{3+} Reduction in Clay Minerals

Bacterial reduction of structural Fe^{3+} has been documented for a variety of clay minerals, including chlorite, illite, nontronite, smectite, and palygorskite, and the process is dominated by the activity of facultative and obligate anaerobic microorganisms (Dong et al., 2009). The extent of bacterially-driven structural Fe^{3+} reduction is dependent upon the clay mineral and microorganism(s) involved, the abundance of Fe^{3+} in the mineral structure, particle surface area, solution chemistry and temperature, relative abundance of bacteria and clay mineral substrate, mineral layer charge, presence of electron shuttles, and the composition of the clay mineral interlayer (Kostka et al., 1999b, Jaisi et al., 2007, Dong et al., 2009). For instance, the reduction of structural iron in clay minerals is related to layer expandability (and inherently, layer charge), such that expandable clay minerals of low layer charge are highly susceptible to bacterial reduction. This is attributed to the relative ease with which electron shuttles and electron donors can reach Fe^{3+} in the mineral structure (Dong et al., 2009). Kostka et al. (1999a) reports that, as a result of bacterial activity, up to 90% of available Fe in swelling smectite clay minerals exists as Fe^{2+}. Supporting that the involved microorganisms influence the extent of microbial iron reduction, Kashefi et al. (2008) reports a 6.3-15.1% reduction of available Fe^{3+} in smectite by thermophilic and hyperthermophilic microorganisms, including *Geoglobus ahangari* and *Geothermobacterium ferrireducens*; this is significantly lower (by roughly 20-50%) than the extent of reduction induced by mesophilic iron reducing bacteria (see Kashefi et al., 2008 and references therein). Kostka et al. (1999a), Kim et al. (2005), Stucki & Kostka (2006), and others have shown that microbial reduction of structural Fe^{3+} can induce numerous changes in the physical and chemical properties of clay minerals, and Dong et al. (2009) and references therein states that the oxidation state of Fe in clay minerals controls swelling, dispersion, flocculation, surface area, reactivity, hydraulic conductivity, and CEC properties of clay minerals.

Kostka et al. (1999a) and Stucki & Kostka (2006) identified that as microbial reduction of structural Fe^{3+} in the octahedral layer of smectite increased, CEC increased, specific surface area decreased, swelling in aqueous solution decreased, smectite dissolution increased, and reactivity with organic compounds increased. Particle surface area of reduced smectite was observed to decrease by 26-46% in comparison to the pre-bioreduction surface area, which induced an increase in particle surface charge density (Kostka et al., 1999a). Alteration of the physical and chemical properties of smectite will cause changes in the adsorptive capacity of the mineral. Stucki & Kostka (2006) noted that the physical and chemical changes observed in reduced smectite were not entirely reversible; that is, upon re-oxidation of reduced smectite, the clay mineral did not retain its pre-reduction physical and chemical properties in their entirety. Effects of structural Fe^{3+} reduction on smectite swelling was predicted by Stucki et al. (2002) and Stucki & Kostka (2006) to depend upon the balance of attractive force between layers (i.e. enhanced attraction between layers caused by the increased number of interlayer cations required to charge balance reduced smectite, van der Waals forces, etc.), and the hydrophilic properties of the basal surfaces (i.e. increased basal surface hydration energy, enhanced Brønsted basicity, enhanced H-bonding).

Kim et al. (2005) investigated the influence of microbial Fe^{3+} reduction on smectite clay flocculation, and found that structural iron reduction significantly alters the electrochemical properties of the clay mineral; namely, reduction causes an increase in the net negative charge exhibited by clay mineral particles, and this, coupled with the production and excretion of microbial EPS, was found to encourage flocculation of suspended clay mineral particles. Alteration of particle charge increased the electrochemical attraction between smectite particles in suspension, which thereby induced flocculation. Thus, clay mineral flocculation is highly dependent upon the redox state of the minerals in suspension, and is inherently driven by microbial activity. The role of EPS in clay flocculation was effectively that of a binding agent, which enabled polymeric bridge formation between clay particles in suspension. In doing so, the attractive force between clay mineral particles was increased, which allowed for the formation of more coherent clay mineral flocs. (Kim et al., 2005).

Kostka et al. (2002) compared the growth of the iron reducing bacterium *Shewanella oneidensis* strain MR-1 in the presence of either smectite or poorly crystalline iron oxyhydroxides as the sole iron source, and observed similar rates and extents of microbial growth in each case. The authors state that mineral surface area was the dominant control in regulating the extent of iron reduction and resultant microbial growth, and that the high surface area of smectite clay minerals is conducive to enhanced microbial reduction and growth. This study supports that iron oxyhydroxides are not preferentially reduced over clay minerals, and that smectite clay minerals require appreciation as iron sources in environmental systems due to the presence of structural Fe^{3+} substitutions in the clay mineral lattice. The ubiquity of iron-bearing clay minerals in environmental systems supports that processes such as structural iron reduction of these minerals, and the correspondingly induced changes in mineralogical and geochemical properties as discussed in the previous paragraphs, likely assume a significant role in the biogeochemical cycling of elements, nutrients, and contaminants. (Kostka et al., 2002).

Mailloux et al. (2009) suggested that the weathering of mineral phases, including apatite, biotite, hornblende, and feldspars can encourage the release of As from the mineral lattice, noting that As release occurred independently of redox conditions, and can occur via reduction of Fe^{3+} in mineral phases, or via fermentation and methanogenesis. Although clay minerals were not directly examined by Mailloux et al. (2009), this study proposed that Fe^{3+} reduction can allow for the simultaneous release of toxic elements, including As, from the mineral lattice. Additional research is needed to evaluate potential links between the release of toxic elements from clay minerals coupled to Fe^{3+} reduction, and investigate subsequent implications of any such toxic element release from clay mineral substrates on the structure of the involved microbial community.

The research of Kim et al. (2005) and Stucki & Kostka (2006) importantly suggests that microbial Fe^{3+} reduction will alter metal uptake by smectite clay minerals, through the balance between altered flocculation and CEC characteristics. Clay mineral flocculation is predicted to negatively affect the ability of clay mineral particles to adsorb metal cations, as enhanced flocculation would cause a decrease in the reactive surface area in contact with aqueous solution. This decline in surface area is predicted to correspond to a decrease in the number of active surface sites available for metal adsorption, and therefore may cause a decrease in the adsorptive capacity of the suspended clay minerals. Further, they postulated that a decrease in specific surface area, coupled with subsequent collapse of smectite layers, will impose changes to clay mineral particles including decreased exchangeability of interlayer cations. Contrarily, the improved CEC resulting from an enhancement in structural Fe^{3+} reduction in smectites as reported by Stucki & Kostka (2006) will permit an increase in the ability of clay mineral particles to adsorb metals. The increase in CEC results from increased layer negativity caused by the addition of electrons to the mineral structure, which thereby increases the affinity of cations towards the clay mineral surface. Therefore, the influence of structural Fe^{3+} reduction on metal sequestration by smectite cannot be simply predicted as it will reflect the balance existing between the negative influence of flocculation on metal sequestration, as shown in the work of Kim et al. (2005), and the positive influence of enhanced CEC induced by reduction of structural iron, as shown in the work of Stucki & Kostka (2006). In conclusion, microbial reduction of structural Fe^{3+} causes numerous physical and chemical changes to mineral constituents, and is postulated to affect the metal scavenging properties of clay minerals. The literature suggests that the degree of influence that structural Fe^{3+} reduction will have on metal uptake by clay minerals will depend on the relative extent of Fe reduction and corresponding clay mineral property alterations (e.g. enhanced flocculation versus enhanced CEC); this, however, remains a testable hypothesis currently unanswered in the literature.

1.5 Bacterial Metal Immobilization

1.5.1 Introduction to Bacterial-Metal Interactions

Biodegradation, a common remediation approach for organic contaminants, is typically not a viable approach for metal cations, as the generation of the free metal typically increases mobility, bioavailability and toxicity. However, speciation and oxidation state, and thus mobility, solubility, bioavailability, and toxicity of metallic contaminants in aqueous environments can be regulated by the activity of microorganisms (Lovley & Coates, 1997, Barkay & Schaefer, 2001). Many species of prokaryotes have developed mechanisms which allow them to cope with toxic metals and further reflect the lengthy co-existence of such species with metals in the environment (Valls & de Lorenzo, 2002, Kosolapov et al., 2004). These mechanisms can directly and indirectly control the fate of metals and metalloids in the environment (Kosolapov et al., 2004). Numerous mechanisms of metal immobilization, including precipitation/biomineralization, biosorption, and intracellular sequestration, are attributed to the presence and activity of microorganisms, and these mechanisms can induce the transformation of bioavailable, aqueous metal species into insoluble, non-bioavailable, and ultimately, chemically inert forms (Lovley & Coates, 1997, Gadd, 2004, Kosolapov et al., 2004). Similarly, numerous mechanisms of metal mobilization are also attributed to microbial activity; examples include chelation, complexation, protonation, leaching, methylation, oxidation state changes and volatilization (Lovley & Coates, 1997, Gadd, 2004, Kosolapov et al., 2004). Microbial controls on metal speciation can greatly affect the geochemical cycling of metals and other elements (e.g. C, P, N, S), and thus, the opportunities to use microbes that influence toxic metal cycling to significantly improve environmental remediation efforts are profound (Lloyd & Lovley, 2001, Gadd, 2004). Environmental remediation efforts utilizing biological entities offer operational flexibility (i.e. utility both *in situ* and *ex situ*) and selectivity in metal uptake (Lloyd & Lovley, 2001), and thus, significant improvements to remediation efforts may arise from the coupling of factors favouring bacterially-attributed metal immobilization with enhanced metal uptake by clay mineral substrates. The remediation of metallic contaminants in aqueous systems is typically addressed via one of three mechanisms: 1) concentration of contaminants into a reduced volume; 2) concentration of contaminants into a reservoir of higher tolerance to effectively reduce harmful impacts; or 3) the precipitation of metallic contaminants, which can often be tightly coupled with microbial activity (Barkay & Schaefer, 2001). Bacterial metal immobilization mechanisms have been reviewed previously (e.g. Barkay & Schaefer, 2001, Valls & de Lorenzo, 2002), but a brief synopsis will be provided here.

1.5.2 Precipitation/Biomineralization

The biomineralization of a wide variety of minerals, including those specifically incorporating cationic metal phases as well as those that are metal-reactive, by a diverse set of metabolisms is possible in the environment. Virtually all groups of microorganisms can mediate, whether directly or indirectly, the formation of minerals (Gadd, 2010). Biomineralization refers to the formation of mineral precipitates attributed to microbial influence, and can proceed authigenically or diagenetically, with mineral precipitation occurring extracellularly, intracellularly, and epicellularly (Ehrlich, 1999, Barkay & Schaefer, 2001, Valls & de Lorenzo, 2002, Konhauser, 2007). Numerous requirements, both physical and chemical, must be fulfilled to permit the formation of stable mineral phases, including: 1) appropriate pH and redox conditions for the given mineral precipitate; 2) dissolved excess of at least one of the mineral-forming components (i.e. solution saturation); and 3) low solubility of the particular mineral phase under prevailing geochemical conditions (Ehrlich, 1999). The relative importance of biotic in comparison to abiotic mineralization is dependent upon predominant geochemical, mineralogical, and microbial characteristics, and current literature identifies microbially-induced formation of metal sulphide, hydroxide, carbonate, and phosphate minerals associated with dissimilatory reduction (e.g. of Fe^{3+}, SO_4^{2-}), templating of minerals, whereby the microorganism and/or EPS serve as nucleation sites for biomineral precipitation, as well as through interactions of metals with metabolic by-products (Ehrlich, 1999, Barkay & Schaefer, 2001, Lloyd & Lovley, 2001, Valls & de Lorenzo, 2002, Gadd, 2004, Kosolapov et al., 2004, Umrania, 2006).

In freshwater aquatic environments, which are significantly under-saturated with regards to most metal minerals (e.g. metal oxides), the only real possibility for metal precipitation through mineralization is associated with sulfide formation, constraining this process to anoxic, sulfidic and typically organic-rich sediments. In general, dissimilatory reduction involves cationic metal transformation, which proceeds independently of metal uptake by the microbe, and thus, the transformed metal commonly remains in the extracellular media (Valls & de Lorenzo, 2002). The dissimilatory reduction of metals is catalyzed by membrane-bound enzymes, which transform metals via oxidation, alkylation, and methylation. Dissimilatory sulphate reduction (by environmentally ubiquitous sulphate-reducing bacteria, SRB) and commonly associated metal sulphide precipitation is typically regarded as the most significant bacterially-driven mechanism in the context of metal immobilization. These processes occur in organic, sulphur-rich environments (e.g. wetland and anoxic organic sedimentary environments), and involve the formation of H_2S through SRB activity and the subsequent precipitation of cationic metal (e.g. Fe^{2+}, Cd^{2+}, Cu^{2+}) sulphides (e.g. Ehrlich, 1999, Kaksonen & Puhakka, 2007, Neculita et al., 2007). The SRB-catalyzed reduction of sulphate generates a net increase in ANC at higher pH values (Equation 1.1) or consumes net protons at pH values lower than pK_{a1} for H_2CO_3 and pK_{a1} for H_2S (Equation 1.2), inducing an increase in pH (Equations 1.1 and 1.2), which can encourage further metal immobilization via the precipitation of metal hydroxides (Gadd, 2004, Kosolapov et al., 2004).

Circumneutral pH (above pK_{a1} for H_2S (7.2 at 25°C)):

Eq. 1.1 $2CH_2O + SO_4^{2-} \rightarrow 2HCO_3^- + HS^- + H^+$

Lower pH values (~4.5-5; $H_2CO_3 \rightarrow H_2O + CO_2$):

Eq. 1.2 $2CH_2O + SO_4^{2-} + 2H^+ \rightarrow 2CO_2 + H_2S + 2H_2O$

One important limitation to the bacterially-induced precipitation of metal sulphides, however, stems from the non-specificity of sulphide precipitation which can result in limitation of bioavailable elements such as Fe, providing a negative feedback on microbial activity (Barkay & Schaefer, 2001). Alternatively, precipitation can also occur as an indirect result of microbial metabolic processes (e.g. precipitation driven by micro-environmental changes facilitated by the excretion of metabolites, etc.) (Valls & de Lorenzo, 2002). The Gram-negative bacterium *Ralstonia eutropha*, for instance, has been shown to precipitate metals as insoluble carbonate biominerals that accumulate extracellularly due to the active bacterial uptake of protons, which induces the formation of micro-environmental zones of high pH surrounding the cell (Lloyd & Lovley, 2001).

However, biomineralization in both anaerobic and aerobic environments can affect subsequent metal scavenging through the formation of metal reactive phases and/or metal precipitated phases (e.g. bioreductive precipitation of Cr(III); Chung et al., 2006). For instance, microbial cell surfaces and biofilms (cells and associated EPS) function as efficient nucleation sites for the precipitation of biominerals, and microbial exudates have also been found to contain precipitated metals (Lovley & Coates, 1997, Barkay & Schaefer, 2001, Lloyd & Lovley, 2001). Biofilms in particular are found to strongly encourage biomineralization as a result of enhanced microbial activity, large biomass development, occurrence of EPS, formation of microenvironments within the biofilm matrix, the ability of microorganisms within a biofilm to withstand extreme environmental conditions, the occurrence of high localized solute concentrations within the matrix, and regulated pH and Eh conditions within the biofilm (Barkay & Schaefer, 2001, Valls & de Lorenzo, 2002, Malik, 2004).

As mentioned previously, numerous environmental parameters can affect the relative dominance of biomineralization over abiotic mineralization. However, biomineralization is discernible under bulk conditions that are thermodynamically favourable but kinetically slow (Amores & Warren, 2007). Of relevance to this review, reflecting their importance in metal sorption, bacterially-mediated Fe-hydroxide/oxyhydroxide mineral precipitation, through nucleation, templating, and/or collection on cell/EPS surfaces, tends to dominate over abiotic Fe-oxyhydroxide precipitation across a broad spectrum of geochemical and environmental conditions in aquatic systems; however, for the most part this phenomenon occurs within aerobic (i.e. water column) environments where Fe^{3+} minerals are stable (see Konhauser, 1998 for review).

Precipitation of toxic metals and metalloids can be viewed as a plausible solution to both effectively remove metal(loid)s from solution and to reduce metal and metalloid toxicity; arsenic removal, for instance, can occur in anaerobic environments characterized by enhanced sulphate reduction and As^{5+} reduction, allowing for the precipitation of insoluble As_2S_3 (Lovley & Coates, 1997, Barkay & Schaefer, 2001). In summary, biomineralization represents a highly effective mechanism by which metals and metalloids can be transformed by biological activity into less toxic, less bioavailable, and less mobile forms.

1.5.3 Biosorption and Intracellular Sequestration

Biosorption refers to the passive, metabolism-independent immobilization of organic- and inorganic metal and radionuclide species onto surface functional groups (e.g. amino, carboxyl, hydroxyl, imidazole, phosphate, sulfhydryl, sulfonate, etc.) of live or dead microbial biomass via physicochemical mechanisms including adsorption, coordination, electrostatic and hydrophobic interactions, ion exchange, and micro-precipitation, and can further encourage larger-scale mineral precipitation with biosorbed cations serving as nucleation sites for subsequent growth (Volesky, 1987, Lovley & Coates, 1997, Gupta et al., 2000, Barkay & Schaefer, 2001, Lloyd & Lovley, 2001, Valls & de Lorenzo, 2002, Gadd, 2004, Kosolapov et al., 2004, Malik, 2004, Gadd, 2009). Despite the passive (i.e. metabolically-independent) nature of biosorption, microorganisms are able to concentrate metals to significantly higher levels than those observed in the surrounding environment (Kosolapov et al., 2004), and as a result, biosorption as a mechanism of metal uptake has been extensively studied and reviewed (e.g. Volesky & Holan, 1995, Chang et al., 1997, Veglio & Beolchini, 1997, Pagnanelli et al., 2000, Sar et al., 2004). Metabolic activity of living cells can strongly influence biomass sorptive properties as a result of metabolically-induced fluctuations in Eh, pH, metabolite presence, and nutrient content (Gadd, 2004). The effectiveness of a particular substrate to function as a biosorbent is dependent upon the composition of the cellular material, the ionic state of the biomass (which can be altered via pre-treatment such as H^+ or Na^+ saturation), the solution characteristics of the metal sorbate, and the physicochemical characteristics of the particular system (e.g. pH, Eh, temperature, ionic strength, presence of ligands), and current research has identified that biosorbed metals largely concentrate in the cell wall, EPS/capsular materials, and the cytoplasm (Volesky, 1987, Gupta et al., 2000, Malik, 2004). Under conditions of given temperature, for instance, metal uptake onto biomass will occur until reaching a state of equilibrium with the surrounding aqueous media (Volesky, 1987). When considering the use of live microbial biomass as the substrate for biosorption, system performance will additionally reflect the availability of nutrients and micronutrients, the physiological state and age of the cells, and various environmental conditions including pH, temperature, and abundance of co-ions (Volesky, 1987).

Biosorption is described by numerous authors as a highly effective metal immobilization mechanism, even from dilute solutions, and features low operational costs while generating minimal quantities of chemical or biological wastes (Gupta et al., 2000, Leung et al., 2000, Lloyd & Lovley, 2001). Biosorbents offer the advantages of selectiveness towards divalent and multivalent metal cations, simple regeneration, inexpensiveness, and efficiency in comparison with commercial adsorbents and ion exchange resins (Volesky, 1987); however, despite the ability of biosorbents to temporarily immobilize metallic contaminants (Lovley & Coates, 1997), the stability of biosorbed metals is regarded by some authors as short-term, due to the relatively short life span of microorganisms (Kosolapov et al., 2004). Both suspended and immobilized biomass (e.g. within bioreactors, as bacterial biofilms on inert support structures, etc.) have undergone evaluation as potential biosorbents, with immobilized biomass typically favoured as a result of easier separation of biomass and liquid components, increased metal tolerance for live cells, and higher mechanical strength (Gadd, 2004).

Leung et al. (2000) showed that biosorption was less effective under acidic conditions, reflecting increased competition for surface sites with H^+ ions at low pH. A strong positive correlation was observed between pH and metal biosorption over the pH range from 2 to 6, and metal uptake has been observed onto cell walls and other external surfaces (e.g. EPS, which is described as a highly effective and selective scavenger of metals) (Leung et al., 2000, Gadd, 2004, Singh & Cameotra, 2004). Competitive sorption between numerous cationic metal species was shown by Leung et al. (2000) to alter the efficiency of biosorptive metal uptake; for instance, when Cu^{2+} was present in solution, decreased Pb^{2+} uptake was observed, which was explained by the authors to result from overlap in preferred sorption sites. Research more specifically targeting the competitive adsorption of metals onto microbial cell surfaces may provide insights into mechanisms that can preferentially enhance adsorption of certain cationic species over others, perhaps through chemical alteration of the adsorbent.

Alternatively to the accumulation of metals onto exudates, extracellular polymeric substances, and other extracellular components of cells, metals can also be sequestered intracellularly. The active intracellular sequestration of metals is referred to as bioaccumulation and is often accomplished through assimilatory metabolic processes (i.e. anabolism) (Gupta et al., 2000, Liermann et al., 2007). Through various anabolic pathways, cationic metals can be sequestered within microbial cells, with the anabolic incorporation of metals requiring introduction to the cell via membrane transport systems and subsequent incorporation into the cell via precipitation, binding or localization within organelles, enzymes and other cell materials (Gadd, 2004, Liermann et al., 2007). However, intracellular sequestration is regarded as less appropriate for use in bioremediation efforts than biosorption as a result of the energy-dependent nature of intracellular sequestration (Sar et al., 2004); thus bioaccumulation will therefore not be discussed further in this review.

Cumulatively, the literature highlights the widespread interaction of microbes with minerals and metals, illuminating the opportunities to use biological approaches for metal remediation. However, the current applied research also shows that the challenge of long-term efficacy remains with regards to biological treatment approaches. These challenges reflect the need to understand more clearly how microbial communities, rather than single strain approaches, affect processes of interest and particularly, the need for greater efforts to constrain the evolution of microbial-mineral assemblages and associated impacts on contaminant sequestration.

1.6 Clay-associated Microorganisms

1.6.1 Characterization of Clay-associated Microorganisms

Although significant research has examined microbial communities associated with aquifer and other higher-permeability geologic units (e.g. Kölbel-Boelke et al., 1988, Ekendahl et al., 1994, Onstott et al., 1998, Green & Scow, 2000, Rogers & Bennett, 2004, Goldscheider et al., 2006, Lehman, 2007), the characterization of microorganisms associated with clay deposits and other low-porosity sediments and soils has received much less attention to date. This fact reflects the numerous unique challenges that arise when attempting to isolate and characterize clay-associated microorganisms, and consequentially, a relatively limited number of related publications exist in the scientific literature (e.g. Haveman et al., 1995, Boivin-Jahns et al., 1996, Lawrence et al., 2000, Fukunaga et al., 2005, Shelobolina et al., 2007, Poulain et al., 2008, Carson et al., 2010). Despite the low abundance of published studies in this area, numerous limitations of presently employed methodological approaches have been identified when applying these techniques to the characterization of clay-endemic microbes.

Scanning electron microscopy (SEM) and epifluorescence microscopy are commonly used to identify microorganisms and observe microbe-substrate interactions in environmental samples. Both techniques, however, have found restricted effectiveness in observing bacteria associated with clay mineral matrices (Boivin-Jahns et al., 1996, Lawrence et al., 2000). The inability to resolve clay-hosted bacterial cells via epifluorescence microscopy or SEM results from the abundance of reflective and autofluorescent mineral grains within clay matrices, the requirement for large dilutions, and the presence of mineral grains of comparable size to bacteria (Lawrence et al., 2000). Haveman et al. (1995) identified issues with the commonly applied fluorescent probes DAPI and acridine orange when used against fine-grained geologic media; for instance, a discrepancy in cell count when cells were enumerated using DAPI versus acridine orange probes was reported, such that cell counts obtained using DAPI were more than twice those determined by acridine orange. These concerns are largely supported by Kostka et al. (1999b), who report inconclusive enumeration of bacterial cells against clay mineral matrices when using DAPI and acridine orange stains; inability to directly enumerate cells requires the use of indirect evidence (for instance, respiration coupling to growth, as in the work of Kostka et al. (1999b)) to support enhancement of microbial growth. Given the numerous issues associated with more commonly applied fluorescent probes, Fukanaga et al. (2005) utilized the fluorescent probe carboxyfluorescein diacetate acetoxymethyl ester (CFDA-AM) to detect the presence of living cells within bentonite clay samples. The authors confirmed the absence of artefacts associated with DAPI and acridine orange probes when using CFDA-AM by analyzing an autoclaved sample as a negative control, which returned a cell count of zero; this lead the authors to support the reliability of this probe in applications examining similar geologic media.

Current methodological limitations associated with commonly applied probes and microscopy techniques lend support to the inherent importance of developing and enhancing new fluorescent probes and techniques appropriate for observing microbial cells affiliated with clay mineral matrices. Greater success was reported by Maurice et al. (2001b) who used atomic force microscopy (AFM) to examine kaolinite grains within a bacterial matrix from microbial growth experiments, supporting the utility of this powerful technique to resolve clay mineral-microbial associations (see Maurice, 1996 for review of environmental applications of AFM). Perhaps additional constraints on clay mineral-microbial interactions will arise from the advancement and application of techniques such as biological force microscopy (BFM), a technique used to measure the attractive and repulsive forces between mineral and bacterial surfaces at distances from 0-2μm (Lower et al., 2001). Lower et al. (2001) proposed that BFM may find utility in understanding processes such as mineral dissolution, attachment of bacterial cells to mineral substrates, and biofilm development; the noted potential of BFM supports further investigations utilizing this technique to examine clay mineral-microbe-metal interactions.

Cultivation-independent studies using polymerase chain reaction (PCR) for the genetic identification of bacterial DNA in clay samples have been reported (Boivin-Jahns et al., 1996, Lawrence et al., 2000). PCR was found to successfully identify bacterial DNA in clay samples at all depths within cored sections, and was used to support the notion that a decline in cell count density is observed with increasing depth in undisturbed clay deposits. However, the direct extraction of bacterial DNA from clay sediments was identified by Poulain et al. (2008) as highly problematic due to low bacterial cell densities and their inhomogeneous distribution within clay matrices reflecting heterogeneity in porosity and mineralogy. Thus, the limited literature characterizing clay associated microbial communities likely underestimates their occurrence, diversity, and density, and therefore the full scope of how they may influence clay characteristics and clay-metal interactions remains to be described.

In addition to the use of genetic, DNA-based characterization, Lawrence et al. (2000) specifically identified through carbon utilization studies that cells of coccoid morphology dominated (~61%) at depth in an undisturbed clay deposit, although rod-shaped bacteria were also common (~39%); those of spiral morphology were absent. In addition, Gram-negative bacteria were shown to dominate the clay unit (~97%) in comparison to Gram-positive (~3%), and numerous species of aerobes, actinomycetes, sulfur oxidizers, methanogens, and other anaerobic species were identified (Lawrence et al., 2000). Bacterial activity and cell density were found by these authors to decrease with depth in undisturbed clay, most likely resulting from the entrapment of bacteria within the highly-compact clay matrix, where exceedingly small pore spaces and throats greatly reduce the mobility of entrapped bacteria (Boivin-Jahns et al., 1996). Pore and throat size assume major importance in the ability of microorganisms to inhabit and move through subsurface geologic materials (Rebata-Landa & Santamarina, 2006). Reduced mobility of bacteria at depth within a clay deposit indicates that microorganisms present are likely those deposited simultaneously with the clay sediments, and/or are descendents of bacteria present at the time of deposition, with variation in community structure reflecting changes in depositional regime or source material, or introduction during subsequent geological events (Lawrence et al., 2000).

1.6.2 Environmental Controls on Clay-associated Microorganisms

Bacteria face numerous challenges living in subsurface clay deposits, and several physical mechanisms have been identified which can result in the death of subject microorganisms (Rebata-Landa & Santamarina, 2006). Among these are: a) puncture, describing the impalement of bacterial cells by clay particles pressed at an angle into the cell wall; and b) tensional breakage, occurring when the space around a bacterial cell is reduced due to compression, causing axial deformation and tensional rupture of the cell wall (Rebata-Landa & Santamarina, 2006). Fukunaga et al. (2005) identified that a decline in moisture content at depth within bentonite clay may explain the observed decrease in bacterial survival, likening the low level of bacterial activity observed in these environments to that previously characterized in desert environments. Bacterial survival in fine grained matrices also depends upon the diffusion-controlled transport of nutrients and waste products through the surrounding geologic media (Boivin-Jahns et al., 1996, Lawrence et al., 2000, Rebata-Landa & Santamarina, 2006). Entrapped bacteria within clay deposits have thus adapted to undergo extremely slow cell division, to exist in low cell count densities, and to survive under nutrient-poor diffusion-controlled conditions by drastically reducing metabolic activity (Boivin-Jahns et al., 1996, Lawrence et al., 2000). These adaptations explain the difficulties encountered in culturing clay-endemic bacteria using standard culture techniques; i.e. nutrient-rich media used in culturing procedures provide extremely different nutrient conditions than those to which target bacterial species are adapted (Boivin-Jahns et al., 1996). In fact, <0.1% of the bacterial cells detected via phospholipid fatty acid (PLFA) analysis of numerous fine-grained sedimentary matrices (shale, siltstone, and argillaceous carbonate) were successfully cultured in the laboratory (Onstott et al., 1998). This fact necessitates the use of specialized media when culturing clay-endemic bacteria; for instance, R2A agar (Reasoner & Geldreich, 1985), which has found use in the culturing of bentonite-associated microorganisms (e.g. Fukunaga et al., 2005 and references therein). Additional constraints found to affect the survival of bacteria in clay deposits include variation in porewater chemistry and the presence of coarser-grained refuges within massive clay deposits (Lawrence et al., 2000), as well as the increasing evidence that environmental microbes are often cooperative, requiring diverse communities for their survival (e.g. Moons et al., 2009). Importantly, the degree of microbial diversity defining a clay deposit is heavily dependent upon the extent of confinement experienced within the particular environment (Boivin-Jahns et al., 1996). Collectively, the emerging research indicates likely greater abundance and diversity of clay-associated microbes than currently identified occur in the environment, with as yet unidentified impacts for clay-metal interactions.

Carson et al. (2010) evaluated the influence of pore connectivity on bacterial diversity in soils, and found that low pore connectivity, corresponding to low water potential/content, induced an increase in the observed bacterial diversity. Under conditions of low water potential and high clay and silt content, a soil is expected to exhibit low effective porosity, which can induce a decrease in bacterial mobility and substrate diffusion within the soil media. The authors found that as pore connectivity is decreased by lowering water potential, the bacterial community within the soil system experiences a lesser degree of competition; reduced competition favours coexistence, and thus, bacterial diversity is enhanced under conditions of low pore connectivity resultant from low water potential. Reduced competition in this case may result from, among other things, the limitation of substrate diffusion to bacterial cells under conditions of low water potential; limited substrate diffusion will result in growth and activity inhibition in bacteria, which may be found to impede the effects of more competitive bacteria on less competitive bacteria. Similarly, if mobility through the soil media is limited under low water content/unsaturated conditions, depletion of limited substrate resources by highly motile bacteria may be inhibited, thereby encouraging diversity. Soil textural influences on bacterial diversity were also investigated by adjusting the relative proportions of clay- and silt-sized particles of quartz in a sandy quartz soil. Bacterial diversity remained constant across the texturally-varied soils, but community structure was found to differ greatly in response to alterations in textural parameters. (Carson et al., 2010 and references therein). Despite the obvious chemical differences that can be expected to exist between clay deposits and sandy quartz soil, this study has likely implications for the physical controls on clay-associated bacterial communities, as nutrient and waste transport in clay deposits is diffusion-controlled (Boivin-Jahns et al., 1996, Lawrence et al., 2000, Rebata-Landa & Santamarina, 2006).

Significant room for advancement through the quantitative and qualitative assessment of microbial diversity in clay deposits exists, and can be achieved through enhancement of current microscopy techniques such as AFM and BFM, as well as the development of: a) novel microscopy techniques sufficient to resolve microorganisms hosted in clay mineral matrices; and b) more effective DNA extraction techniques that are better suited for use on clay sediments than those presently employed.

1.7 Future Research

Research efforts in recent years have provided numerous insights into metal sorption by clays and clay minerals (e.g. Lothenbach et al., 1997, Suraj et al., 1998, Ikhsan et al., 1999, Papini et al., 2001, Bostick et al., 2002, Cooper et al., 2002, Babel & Kurniawan, 2003, Chorover et al., 2003, Chantawong et al., 2003, Yavuz et al., 2003, Usman et al., 2005, Adebowale et al., 2006, Dal Bosco et al., 2006, Potgieter et al., 2006, Abollino et al., 2007, Carvalho et al., 2007, Gu & Evans, 2007, Kubilay et al., 2007, Veli & Alyüz, 2007, Abollino et al., 2008, Bedoui et al., 2008, Bhattacharyya & Gupta, 2008a,b, Chaari et al., 2008, Ribeiro et al., 2008, Akar et al., 2009, Ijagbemi et al., 2009, Li et al., 2009, Sipos et al., 2009). Similarly, significant research has been conducted which examines the immobilization of metals from aqueous solution by bacteria (e.g. Lovley & Coates, 1997, Konhauser, 1998, Gupta et al., 2000, Lloyd & Lovley, 2001, Valls & de Lorenzo, 2002, Kosolapov et al., 2004, Malik, 2004, Sar et al., 2004, Singh & Cameotra, 2004). Finally, research has targeted the interactions occurring between clay minerals and microorganisms (e.g. Bennett et al., 1996, Kostka et al., 1999a, Liermann et al., 2000, Barkay & Schaefer, 2001, Bennett et al., 2001, Edwards & Rutenberg, 2001, Kim et al., 2004, Roberts, 2004, Rogers & Bennett, 2004, Kim et al., 2005, Stucki & Kostka, 2006, Buss et al., 2007, Uroz et al., 2009). However, significant gaps in current scientific literature exist when considering the influences of microbial activity on metal sequestration by clay minerals, and the diversity and characterization of microorganisms endemic to subsurface clay deposits. Minimal research has been conducted which acts to bridge the gap existing between clay mineral-metal adsorption mechanisms and microbial controls on metal mobility in the environment, and thus, abundant room for growth and expansion remains. Observation of dimensionally-induced changes to material properties upon entering the nano-scale lends support to the notion that the ever-growing field of nanoscience and its implications for geoscience-related problems (e.g. Hochella, 2002a,b, Kamat & Meisel, 2003, Hochella & Madden, 2005, Hochella, 2006, Reich et al., 2006, Johnston, 2010) demonstrates extreme potential for further constraining clay mineral-microbe-metal interactions in the environment. Additional research, for instance, investigating the laboratory synthesis and characterization of nanoclays (e.g. El Mouzdahir et al., 2009, Darvishi & Morsali, 2011) and clay mineral-supported nanoparticles (e.g. Üzüm et al., 2009, Yuan et al., 2009) will also assist in this regard. For detailed review of techniques applicable to the characterization of nanomaterials, including X-ray photoelectron spectroscopy, scanning electron microscopy, transmission electron microscopy, powder X-ray diffraction, neutron diffraction, electron energy-loss spectroscopy, and various forms of scanning probe microscopy, among other applicable characterization techniques, see Rao & Biswas (2009). Continued improvements and optimization of these techniques will certainly assist in nanoscale characterization and observation of clay mineral-metal interactions, clay mineral-microbe interactions, and microbe-metal interactions.

Significant advancements in understanding of clay mineral-microbe-metal interactions may arise from research targeting: a) the coupling of microbial immobilization with the use of low-cost clay mineral adsorbents; b) the design and evaluation of bioreactors which incorporate microorganisms with low-cost clay mineral adsorbents; c) pilot- and field-scale studies employing bioreactors derived from research conducted in part b); d) additional case studies of particular clay deposits incorporating adsorption characterization of metals by natural clays from real-world deposits (as opposed to using pure clay mineral standards obtained via mineral synthesis, which have been used in much of the research conducted to date); e) a greater examination of multi-metallic contaminant adsorption, as opposed to mono-species adsorption experiments which dominate the literature (thus enhancing knowledge of competitive adsorption of cationic metals); f) experimentation involving a broader array of metals and metalloids than those focused on in previous experiments, used to further assess the effectiveness of clay minerals as adsorbents for metals with variable properties (e.g. ionic charge, ionic radius, existence as oxyanions, solubility, etc.) and of interest in differently impacted environments or systems; g) assessment of the impact that microbial interactions (e.g. mineral weathering) with clay mineral adsorbents have on the stability of adsorbed metals; h) assessment of the effects upon treatment system longevity (affected via adsorbent dissolution or flocculation, for instance) that microorganisms may potentially have; i) the enhancement and development of novel techniques to allow for more efficient microscopic and other analyses of clay-associated microorganisms, and isolation of microbial DNA from clay matrices; and j) continued advancements in the field of nanoscience targeting clay mineralogy, clay mineral-microbe interactions, microbe-metal interactions, and clay mineral-metal interactions. Improvements to the sustainable treatment of metal-contaminated wastewaters can result from further advancements of current knowledge and understanding, and the strengthening of the aforementioned areas of weakness can greatly enhance the efficiency, longevity, and sustainability of current water treatment strategies (see Figure 1.3).

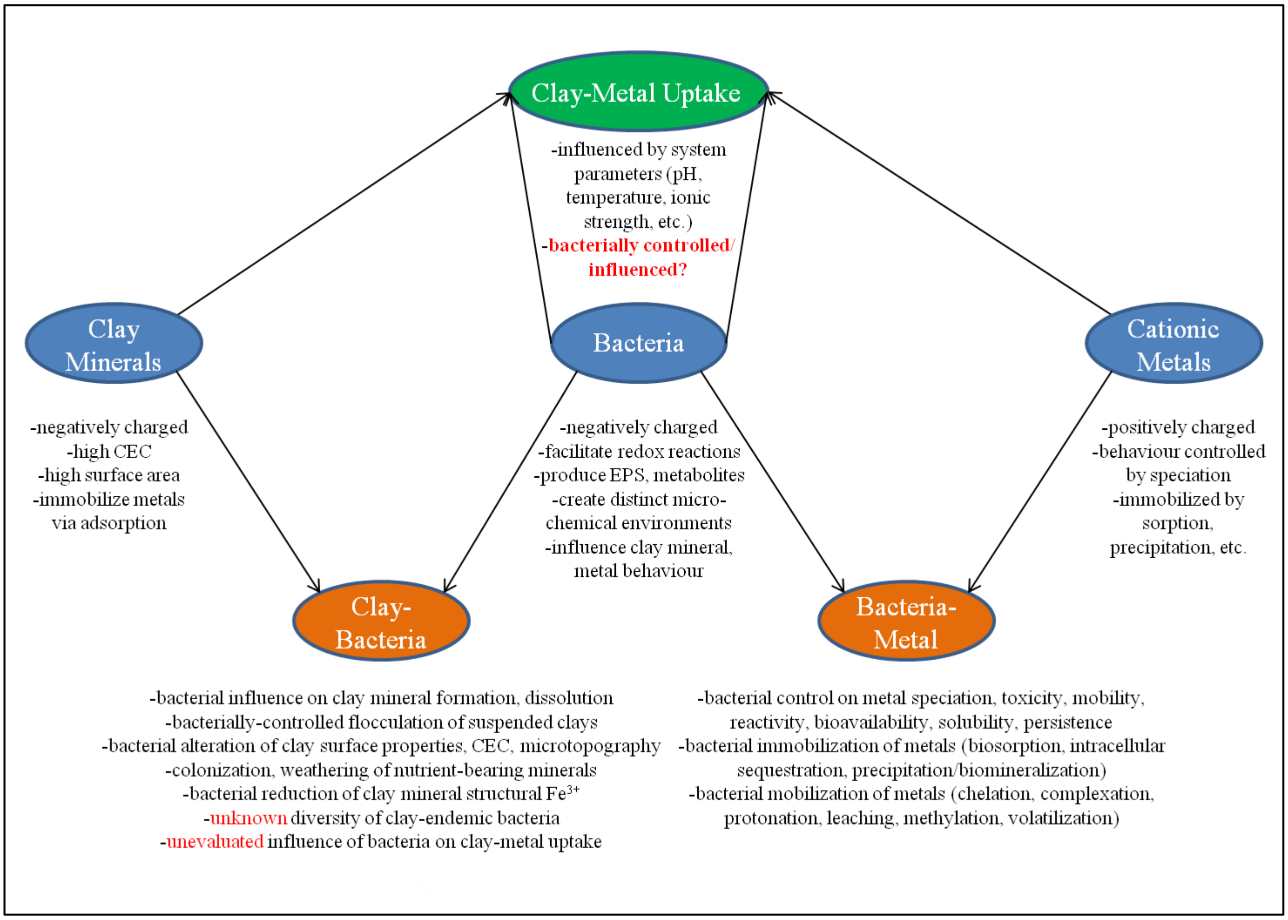

Fig. 1.3 – Schematic of potential clay-bacterial-metal interactions in the environment

2.0 Research Scope

Current scientific literature supports the utility of clays and clay minerals to serve as low-cost sorbents for toxic metals in the environment, and has identified numerous interactions found to occur between clay minerals and iron reducing bacteria. Despite the success of previous experimental work in this regard, current research has yet to resolve a number of important questions pertinent to the use of clay and clay mineral sorbents in the presence of environmentally-ubiquitous iron reducing bacteria. The ability of iron reducing bacteria to influence clay mineral swelling, dispersion, flocculation, surface area, reactivity, hydraulic conductivity, and cation exchange properties, and to promote the dissolution of clay mineral phases, is well documented in the literature (see Section 1.4.5); however, investigation into the influence of iron reducing bacteria on clay and clay mineral sorptive properties, as well as the resultant fate of clay-sorbed metals has yet to occur. This thesis investigates the sorption of toxic metal cations (Cu^{2+}, Cd^{2+}) and a metabolically-available, oxyanion-forming metalloid (As^{5+}) to natural bentonite clay substrates in the presence of iron reducing bacteria enriched from two very different anthropogenically-affected environments – 1) the Athabasca Oil Sands composite tailings; and 2) a base metal mine tailings environment. While the related literature is dominated by abiotic sorption studies typically quantifying metal uptake over short-term (i.e. <24 hour) time scales, this thesis evaluated metal(loid) sorption in the presence of iron reducing bacteria, and sorbed metal and metalloid stability over a six week period. The extended duration over which sorption experiments were conducted in these experiments allowed for elucidation of the longer-term effectiveness of bentonite clays in the presence of iron reducing bacteria in the context of metal(loid) sorption.

The primary research questions and corresponding hypotheses and objectives addressed by this Master's thesis are:

1. What are the influences of potentially metal-tolerant Fe-reducing bacteria on metal(loid) uptake by bentonite clay sorbents of varying smectite content and on the stability of bentonite clay-sorbed metals and metalloids?

Hypotheses:

i. There will be an observable influence of Fe-reducing bacteria on bentonite clay-metal(loid) sorption.

ii. The influence of Fe-reducing bacteria on metal(loid) sorption dynamics will show a negative correlation with bentonite clay smectite content (i.e. decreasing influence with increasing smectite content).

iii. Environmental Fe-reducing bacterial enrichments from the Athabasca Oil Sands composite tailings versus enrichments from a base metal mine tailings environment will show different impacts on bentonite clay-metal(loid) sorption.

iv. Microbially-linked element specificity in metal(loid) sorption and desorption will occur.

Objectives:

a. Evaluate metal(loid) sorption in the presence of Fe-reducing bacteria from two anthropogenically-influenced environments onto three natural bentonite clay samples ranging in smectite content from 15-58%.

b. Assess microbial impacts on long-term bentonite clay-sorbed metal(loid) stability.

2. Are there differences in the influence of Fe-reducing bacteria on Cd^{2+} and Cu^{2+} sorption to bentonite clays when As^{5+}, a reducible, metabolically-available metalloid, is present in solution?

Hypothesis:

i. With As^{5+} amendment, the influence of Fe-reducing bacteria on cationic metal sorption will be diminished.

Objective:

a. Assess Cu^{2+} and Cd^{2+} sorption in the presence and absence of As^{5+} against the aforementioned Fe-reducing bacterial treatments.

Overall, the information gleaned from these experiments will help to resolve the question of whether clay-based sorbents can feasibly be relied upon as permanent treatment mechanisms with the capability of facilitating the long-term sequestration of metals and metalloids, or whether they merely provide a temporary and *microbially-vulnerable* solution for the remediation of metal(loid)s in the environment.

3.0 Methods, Materials and Analytics

3.1 Chemicals, Reagents and Labware Preparation

Appropriate protocols were followed throughout the experimental procedures detailed herein to maintain microbial sterility and trace metal cleanliness, thereby minimizing potential routes of contamination. All reagents used throughout the methodological approaches outlined herein were of reagent grade or higher (Sigma-Aldrich Co., St. Louis, MO, U.S.A.), and trace metal grade nitric acid (HNO_3) was used for sample dilution and solution chemistry related to ICP-MS/OES analysis (see Section 3.5). Aqueous and ethanol-rinse solutions were prepared using 18.2MΩ/cm reverse osmosis deionized Milli-Q water (Millipore, Billerica, MA, USA). Laboratory glass- and plastic-ware was cleaned extensively prior to use; labware was soaked in a phosphate-free detergent bath of Extran 300, and was then scrubbed, rinsed with 70% (v/v) ethanol to remove hydrophobic residues, and rinsed with Milli-Q water. Labware was then immersed in a 4% (v/v) reagent grade HCl acid bath for a minimum of twelve hours to facilitate bound metal removal, and was thereafter rinsed ten times with Milli-Q water. Subsequently, all labware was capped, sealed with parafilm, or enclosed within acid washed plastic bags. Acid-washed labware requiring sterilization was rinsed with a 70% (v/v) solution of ethanol made with Milli-Q water and anhydrous ethanol.

3.2 Bentonite Clay Sample Preparation

Bentonite clay samples utilized in the experiments detailed herein were drill cored, air dried, and collected by Headwater Mineral Exploration and Development, Ltd. from the study deposit located in Swan Hills, Alberta, Canada (see Figure 3.1) in 2008. Prior to the conduction of the experimental procedures detailed below, drill cored segments of bentonite clay samples were homogenized in an acid washed porcelain mortar and pestle and were passed through acid washed 100μm nylon mesh. Representative aliquots of each bentonite clay sample were weighed and dried for 72 hours at 40°C to confirm negligible water content. Confirmatory drying was conducted at low temperature to prevent the removal of interlayer and structurally-bound water molecules from constituent clay minerals, which would thus alter sample mineralogical properties.

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Fig. 3.1 – location of field site in Swan Hills, Alberta, Canada; modified from http://atlas.nrcan.gc.ca/site/english/maps/reference/provincesterritories/alberta/referencemap_image_view; retrieved Jan. 27/10

Once ground and homogenized, bentonite clay samples underwent sterilization via gamma irradiation at the McMaster Nuclear Reactor (McMaster University, Hamilton, Ontario). Gamma irradiation was selected over other methods of sterilization (e.g. autoclaving, microwave irradiation) due to the hydrated nature of constituent clay minerals, the potential for other sterilization methods to alter sample mineralogy (i.e. through the removal of structurally-bound water molecules), and the potential for metal contamination of the clay samples to arise as a result of autoclaving. Following sterilization, samples were dispensed only under sterile conditions within a Class II A/B3 Biological Safety Cabinet (hereafter referred to as BSC; Model 1284, Forma Scientific Inc., Ohio, USA).

3.3 Iron Reducing Bacteria Enrichment Procedure

Complimenting the novelty of the experimental design utilized in this thesis, the incorporation of microbial consortia derived via environmental enrichment into batch sorption experimental microcosms represents an important deviation from the majority of studies examining the influence of Fe-reducing bacteria on metal(loid) mobility and/or geological materials. Predominantly, the literature is comprised of research utilizing pure cultures of known Fe-reducing bacteria, including members of *Shewanella sp.* and *Geobacter sp.* While these studies have produced important results which provide fundamental understanding of the influence of Fe-reducing bacteria in various environmental processes, they are *limited* by a lack of *real-world applicability*; primarily, this is derived from the idea that bacterial species in the environment typically coexist within diverse communities, rather than existing in the absence of other microbial life.

Environmental enrichments of iron reducing bacteria (IRB) were cultured for use in batch sorption experiments from bentonite clay samples, Athabasca Oil Sands composite tailings samples, and base-metal mine tailings samples following the procedures detailed by Kostka & Nealson (1998). Sediment samples from the aforementioned environments were added without pre-treatment under sterile conditions to M1 basal media and enrichments were conducted microaerophilically without agitation on the laboratory bench top. Vessels remained capped with sterile foil to prevent the introduction of foreign bacteria.

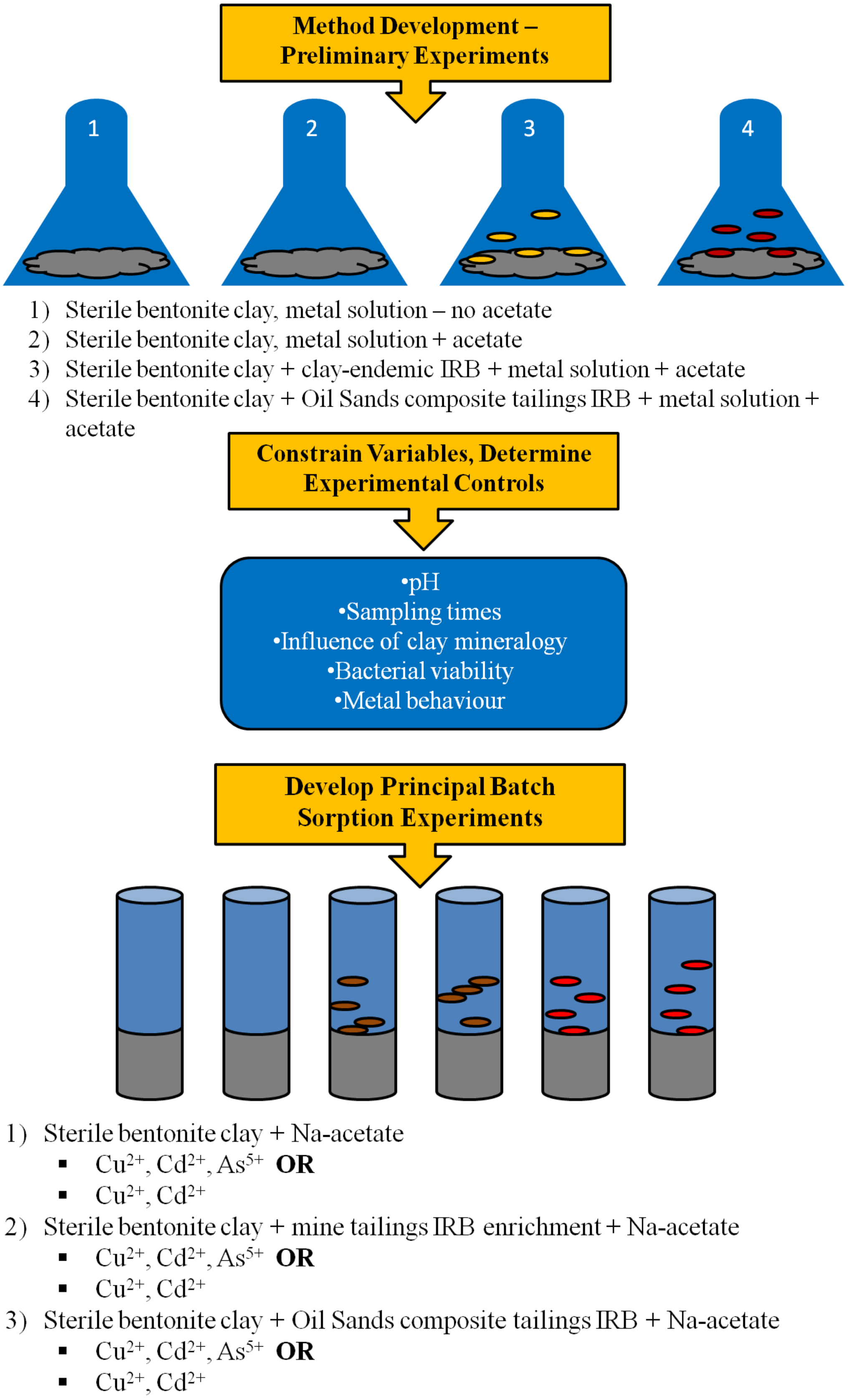
Approximately 5g of the appropriate sediment/tailings material was added to individual Erlenmeyer flasks containing 1000mL of M1 media, and cultures were flipped into fresh media of the same composition periodically. To prevent the proliferation of fungal microorganisms within the environmental enrichments, each 1000mL volume of media was augmented with 10mL of filter-sterilized cycloheximide (20mg/mL stock) and 10mL of filter-sterilized nystatin (5mg/mL stock).

M1 media was prepared according to Kostka & Nealson (1998); variable concentration ranges were provided for several of the media components in this protocol, and only these components will be addressed herein. For full details on media composition, see Kostka & Nealson (1998). Na-acetate was added as an organic carbon source to a final concentration of

10mM. 10mL of the amino acid supplement was provided, and bactoyeast extract was added to a final concentration of ~0.01% (w/v). Finally, Fe(III)-citrate was used as the reducible iron source (final Fe^{3+} concentration of 25mM), and was selected over solid Fe^{3+} sources to prevent surface area limitations from inhibiting bacterial growth. Media augmentation with Fe(III)-citrate accommodates for simple confirmation of the reduction of Fe^{3+} in solution, such that the orange/brown media turns increasingly colourless with enhanced reduction of Fe^{3+} (see Figures 4.18a-d); this allows media colour to serve as an effective proxy for Fe-reducing bacterial growth. Epifluorescence microscopy (LIVE/DEAD® imaging) was used to confirm the presence of live bacteria in enrichments prior to use in batch sorption experiments (see Section 3.7). 16S rRNA gene sequencing (see Section 3.6) was performed on environmental enrichments to determine bacterial community composition.

3.4 Batch Metal(loid) Sorption Experimental Protocols

Given the novelty of the combined biological/abiotic focus of this study which investigated the sorption of toxic metals and metalloids to bentonite clays and the influence of iron reducing bacteria on uptake and sorbed metal(loid) stability, several modifications to classic batch metal sorption experimental protocols were required. Experimental protocols were adapted from the procedures detailed in EPA method 530/SW-87/006, Batch-type procedures for estimating soil adsorption of chemicals (Roy et al., 1992). Observed differences in metal(loid) sorption to bentonite clay substrates in the presence and absence of Fe-reducing bacteria allow for novel understanding of the influence that IRB have on metal(loid) uptake by clay mineral-based substrates. Further, an enhanced understanding of the stability of bentonite-sorbed metals/metalloids is facilitated. Bacterial influences on metal(loid) sorption and stability may arise from microbial alteration of clay mineral surface properties as identified in numerous studies to date (e.g. Kostka et al., 1999, Kim et al., 2005, Stucki & Kostka, 2006, Dong et al., 2009; see Section 1.4 for details). Two sets of batch sorption experiments were conducted throughout this study (see Figure 3.2), with preliminary experiments serving as a means of method development and optimization. Preliminary experiments therefore accommodated for the delineation and appropriate constraining of controls, sampling times, and experimental procedures to be adapted for use in the principal batch sorption experiments.



1) Sterile bentonite clay, metal solution – no acetate
2) Sterile bentonite clay, metal solution + acetate
3) Sterile bentonite clay + clay-endemic IRB + metal solution + acetate
4) Sterile bentonite clay + Oil Sands composite tailings IRB + metal solution + acetate

1) Sterile bentonite clay + Na-acetate
   - Cu^{2+}, Cd^{2+}, As^{5+} **OR**
   - Cu^{2+}, Cd^{2+}
2) Sterile bentonite clay + mine tailings IRB enrichment + Na-acetate
   - Cu^{2+}, Cd^{2+}, As^{5+} **OR**
   - Cu^{2+}, Cd^{2+}
3) Sterile bentonite clay + Oil Sands composite tailings IRB + Na-acetate
   - Cu^{2+}, Cd^{2+}, As^{5+} **OR**
   - Cu^{2+}, Cd^{2+}

Fig. 3.2 – Research Design

3.4.1 Method Development – Preliminary Batch Metal Sorption Experimental Protocols

As noted previously, the primary function of preliminary batch metal sorption experiments conducted in this study was that of method development, such that preliminary experiments were used to develop techniques and procedures to constrain variables employed in principal batch sorption experiments (see Section 3.4.2). Consequently, detailed discussion of experimental results gathered from preliminary experiments will not be presented in this thesis, but important findings, pertinent to the justification of experimental conditions applied in principal batch sorption experiments, will be highlighted.

Preliminary batch metal sorption experiments were conducted on four bentonite clay samples, and the protocols detailed herein were developed to investigate divalent cationic metal uptake (Cu^{2+}, Ni^{2+}, Cd^{2+}) onto bentonite clay substrates and the relative influence of bentonite clay-endemic Fe-reducing bacteria (i.e. Fe-reducing bacterial cultures enriched from the bentonite samples) and Fe-reducing bacteria enriched from the Athabasca Oil Sands composite tailings on metal uptake and sorbed metal stability. The preliminary experiments were conducted under the following conditions for each bentonite clay sample evaluated (provided in brief in Table 3.1): 1) sterilized bentonite clay + metal solution; 2) sterilized bentonite clay amended with organic carbon (Na-acetate) + metal solution; 3) sterilized bentonite clay amended with Na-acetate and clay-endemic IRB + metal solution; and 4) sterilized bentonite clay amended with Na-acetate and IRB from the Oil Sands composite tailings + metal solution. Na-acetate was provided as a carbon source in the IRB enrichment media (detailed in Section 3.3), supporting its use as an organic carbon source in batch sorption experiments.

Table 3.1 – Preliminary batch sorption experimental treatments.

Treatment	**Substrate**	**Solution**	**Organic Carbon**	**Sterile/ Augmented**	**Purpose**
1	Sterile clay	100µM Cu^{2+}, Ni^{2+} and Cd^{2+}	None added	Sterile	Abiotic metal uptake
2	Sterile clay	100µM Cu^{2+}, Ni^{2+} and Cd^{2+}	10mM Na-acetate	Sterile	Abiotic metal uptake in presence of organic C
3	Sterile clay	100µM Cu^{2+}, Ni^{2+} and Cd^{2+}	10mM Na-acetate	Clay-endemic IRB	Influence of clay-endemic IRB
4	Sterile clay	100µM Cu^{2+}, Ni^{2+} and Cd^{2+}	10mM Na-acetate	Oil sands IRB	Influence of potentially metal-tolerant oil sands IRB

Preliminary batch sorption experiments utilized a sorbent: sorbate solution ratio of 1:20; 2.50g of bentonite clay sample was exposed to 50mL of sorbate solution. Solution preparation was accomplished volumetrically by dissolving appropriate quantities of Cu-, Ni-, and Cd-chloride salts (see Table 3.2) into Milli-Q water to achieve an initial concentration of 100µM Cu^{2+}, Ni^{2+} and Cd^{2+}. Metal solutions for Treatments 2-4 were supplemented with Na-acetate to a final acetate concentration of 10mM. All solutions were adjusted to pH 7.00 with 1M NaOH and were vacuum filtered through a 0.2µm membrane into a sterile bottle to ensure microbial sterility while maintaining intended metal loading (which could be altered by sterilization procedures such as autoclaving). Although batch sorption experimental procedures typically utilize metal nitrate salts (e.g. Flemming et al., 1990) or sulphate salts (e.g. Yavuz et al., 2003), chloride salts were selected in this case to prevent the artificial stimulation of microbial activity in response to the presence of excess nitrate or sulphate anions in solution. Aliquots of initial metal solutions were retained and acidified once filtered, analyzed via ICP-MS (see Section 3.5), and represent analyte concentrations at t = 0h.

Table 3.2 – Metal salts utilized in preliminary batch sorption experiment stock solutions

Metal	**Salt**	**Valence**
Cu	$CuCl_2$	Cu^{2+}
Ni	$NiCl_2$	Ni^{2+}
Cd	$CdCl_2 \cdot xH_2O$	Cd^{2+}

Bentonite clay samples were massed and introduced into acid washed, ethanol-sterilized 250mL Erlenmeyer flasks contained within the BSC. 48mL of the appropriate filter-sterilized metal or metal/acetate solution was then added to each vessel. Treatment 1 and 2 flasks were provided with an additional 2mL of Milli-Q water, while Treatment 3 and 4 flasks were provided with 2mL of the appropriate IRB suspension. IRB suspensions were prepared by centrifuging aliquots of IRB-enrichment cultures (see Section 3.3) for 20min at 12 000 x g to pellet out bacterial biomass from the media. The pellet was then washed via re-suspension in filter-sterilized distilled water and was centrifuged again for 20min at 12 000 x g; this washing procedure was repeated twice, after which point, biomass was re-suspended in filter-sterilized Milli-Q water and pH-adjusted to 7.00 with filter-sterilized NaOH or HCl. 2mL of washed bacterial suspension was added to Treatment 3 and 4 flasks. Prior to inoculation of experimental microcosms, LIVE/DEAD® imaging was used to confirm bacterial viability after the washing procedure was conducted (see Section 3.7). In addition to the treatments listed previously, metal solution blanks and metal/acetate solution blanks were run concurrently, consisting of 48mL metal or metal/acetate stock solution + 2mL Milli-Q water. These blanks were used to monitor changes in metal concentration experienced throughout the duration of the experiments (e.g. metal loss via adsorption to the Erlenmeyer flask).

Vessels were topped with sterile foam plugs to accommodate for gas exchange and to create a microaerophilic environment within. Once capped, the flasks were briefly shaken and were then allowed to stand on the bench top until required for sampling. Note that static reaction conditions represent an important deviation from EPA protocols, which call for the use of a bench top rotator. Agitation was avoided to allow the microaerophilic environmental conditions within the flask to persist; microaerophilic conditions were desired to allow for the proliferation of IRB. Six flasks were established for each of the aforementioned treatments, allowing individual flasks of each treatment to be sacrificed at each sampling period (2h, 12h, 24h, 48h, 7d, 1 month). The extended duration of these sorption experiments represents another important deviation from standard sorption experimental protocols, and this deviation reflects the question of bacterial influences on sorbed metal stability. The sampling periods selected allowed for quantitative inferences into metal uptake during the initial, rapid uptake phase characteristic of sorption onto mineral substrates, and the hypothesized time-staggered influence of IRB on metal stability corresponding to metabolically-coupled processes, perhaps resulting in changes in solution chemistry or substrate alteration. Vessels were maintained at ambient room temperature (23 +/-3°C) during the reaction period.

Sampling periods involved obtaining pH (Denver Instrument Model 225 pH ISO Meter, Bohemia, NY, U.S.A.) and temperature within subject flasks. 2.5mL of slurry sample was retained and preserved to 2% glutaraldehyde for potential imaging via SEM-EDS. Once preserved, these samples were stored at 4°C. The remaining slurry sample was centrifuged for 20min. at 12 000 x g and aliquots were retained for (operationally defined) dissolved metals analysis by filtration through a 0.2μm syringe filter. The filtrate was immediately preserved with concentrated trace metal grade HNO_3 to a final concentration of 2% HNO_3 (v/v) and was thereafter stored at 4°C until required for ICP-MS/OES analysis (see Section 3.5). Remaining supernatant was acidified and stored at 4°C with no filtration. Approximately 2.0g of the pellet was retained and frozen at -20°C immediately in case needed for later re-characterization via XRD and/or sequential extraction.

The remaining pellet was preserved for epifluorescence microscopy imaging; preservation involved resuspension in 0.5mL 1xPBS (phosphate-buffered saline) and 4.5mL 4% paraformaldehyde (PFA) for ~16hours. After 16 hours, the samples were centrifuged and the supernatant discarded; the pellet was resuspended in 1mL 1xPBS and 1mL 95% ethanol. 1.5mL of the resuspension was retained and frozen at -20°C until required for imaging (see Section 3.7). Significant difficulties were encountered when attempting to image clay matrix-hosted bacterial cells via epifluorescence microscopy. Difficulties arose from: a) the presence of mineral grains of similar size to bacterial cells; b) considerable matrix autofluorescence and background fluorescence; and c) the requirement for large dilutions to be applied. Such difficulties prevented bacterial viability confirmation at the culmination of these experiments, requiring adaptations to be made for confirmation of bacterial viability in principal batch metal(loid) sorption experiments.

3.4.2 Principal Batch Metal(loid) Sorption Experiment Methodology

Experimental protocols utilized in principal batch sorption experiments were developed and optimized based on observations corresponding to preliminary sorption experiments (see Table 3.5). Preliminary batch metal sorption experiments showed varying results for microbial treatments dependent upon smectite content of the bentonite clay samples, such that remobilization of bound metals was observed after seven days only when smectite was present in lower abundance (e.g. 17-19%). In comparison, no remobilization was observed when smectite was present in greater abundance (~60%). In an effort to further evaluate this finding, principal batch sorption experiments were run on three additional bentonite clay samples of varying smectite content; samples are referred to hereafter in terms of constituent smectite content (high – 58% smectite, medium – 29% smectite, and low – 15% smectite).

Principal sorption experiments utilized a sorbent: sorbate solution ratio of 1:20, whereby 1.25g of sterile bentonite clay sample was exposed to 25mL of pH 4.00 metal(loid) sorbate/Na-acetate solution; note that this pH refers to solution pH prior to the addition of bentonite clay sample. Rather than chemically buffering solution pH, no buffers were added; this allowed the bentonite clay mineralogy and microbial interactions with the substrate and/or solution species to dictate solution pH. Sorbate solution preparation was accomplished volumetrically by dissolving appropriate quantities of Cu- and Cd-chloride salts and arsenate salt (see Table 3.3) into Milli-Q water to achieve an initial concentration of ~100μM Cd^{2+}, Cu^{2+} and As^{5+}. Trace element solutions were pH-adjusted and vacuum filtered through a 0.2μm membrane into a sterile bottle to ensure microbial sterility. Filtrates of the initial metal(loid) solutions were retained and acidified for dissolved metal/metalloid quantification (see Section 3.5), and instrumentally-determined concentrations were used in calculations to determine relative concentrations of metal(loid)s bound in sorption experiment treatments. Sorbate solutions used in principal batch sorption experiments were augmented with 10mM Na-acetate to serve as an organic carbon source.

Table 3.3 – Metal/metalloid salts utilized in principal batch sorption experiment stock solutions

Metal/Metalloid	Salt	Valence
Cu	$CuCl_2$	Cu^{2+}
As	$HAsNa_2O_4 \cdot 7H_2O$	As^{5+}
Cd	$CdCl_2 \cdot xH_2O$	Cd^{2+}

Principal batch sorption experiments investigated the influence of three distinct microbial treatments (sterile, augmentation with IRB from base metal mine tailings, and augmentation with IRB from the Athabasca Oil Sands composite tailings), which were run against two chemical treatments, the first containing ~100μM Cu^{2+}, Cd^{2+} and As^{5+} in 10mM Na-acetate, and the second containing ~100μM Cu^{2+} and Cd^{2+} in 10mM Na-acetate (see Table 3.4).

Table 3.4 – Principal batch sorption experimental treatments

	Treatment 1 (T1)		**Treatment 2 (T2)**		**Treatment 3 (T3)**	
Microbial Treatment	Sterile		Base metal mine tailings IRB		Oil Sands composite tailings IRB	
Chemical Treatment	Cu^{2+}, Cd^{2+}, As^{5+}	Cu^{2+}, Cd^{2+}	Cu^{2+}, Cd^{2+}, As^{5+}	Cu^{2+}, Cd^{2+}	Cu^{2+}, Cd^{2+}, As^{5+}	Cu^{2+}, Cd^{2+}

Clay samples were massed and introduced into acid washed, ethanol-sterilized 50mL centrifuge tubes within the BSC. 24mL of filter-sterilized metal/acetate solution was then added to each vessel. Sterile samples were provided with 1mL of Milli-Q water, while microbial samples were augmented with 1mL of the appropriate IRB suspension (stock IRB suspension dry biomass content: base metal mine tailings IRB suspension → $4.3x10^{-4}$g dry biomass/mL; Oil Sands composite tailings IRB suspension → $4.2x10^{-4}$g dry biomass/mL); for details on IRB suspension preparation and washing, see Section 3.4.1. Once washed, the pellet was re-suspended in filter-sterilized Milli-Q water and was adjusted to pH 4.00 with filter sterilized HCl and NaOH. LIVE/DEAD® imaging was used to confirm bacterial viability after the washing procedure was conducted.

Once augmented with Milli-Q or IRB, sample vessels were capped and vortexed to ensure exposure of bentonite clay sorbent to the sorbate solution. Sample vessels were foil-wrapped throughout the duration of these experiments to discourage the proliferation of photosynthetic microorganisms. These experiments were conducted microaerophillically on the laboratory bench top at ambient room temperature (23 +/-3°C). The sampling schedule established in this set of experiments was applied in an effort to constrain and capture the potential time-sensitive remobilization of metal(loid)s, as observed in preliminary experiments to occur between one week and one month; sampling periods were conducted weekly from one to six weeks, and involved sacrificing one reaction vessel for each microbial/chemical treatment combination (i.e. Treatment 1 with As, Treatment 1 without As, Treatment 2 with As, Treatment 2 without As, Treatment 3 with As, and Treatment 3 without As). pH and temperature were recorded, and the full volume was centrifuged for 20min at 12 000 x g, filtered (0.2μm membrane), and preserved with concentrated trace metal grade HNO_3 to a final concentration of

2% HNO_3 (v/v). Aliquots of the pellet were retained for SEM-EDS, preserved in 2% glutaraldehyde, and stored at 4°C. The remaining pelleted material was stored without further treatment at -20°C for metal(loid) extraction and XRD analysis.

Due to difficulties encountered with epifluorescence imaging of clay-hosted bacteria (see Section 3.4.1), aliquots of slurry were re-suspended in fresh iron reducing media at the conclusion of the six week sampling period. Once turbidity was evident in the media, epifluorescence was used to confirm the presence of viable bacteria (see Section 3.7).

Table 3.5 – Batch sorption experiment method development

Variable/Parameter/ Attribute	Observations in Preliminary Experiments	Modification to Principal Experiments
Sampling schedule	-rapid initial metal uptake and complete immobilization until 7 days; remobilization between 7 days and 1 month in microbial treatments	-staggered weekly sampling periods (1-6 weeks)
Initial solution pH	-initial pH = 7.00; increased up to ~9.3 by conclusion	-adjust initial solution pH to 4.00
Confirmation of bacterial viability	-difficulties with LIVE/DEAD® imaging clay-hosted bacteria prevented confirmation of bacterial viability at the conclusion of experiments	-resuspend aliquot of slurry in fresh Fe-reducing media and image media to confirm viability
Metal behaviour	-very similar behaviour of Cu^{2+} and Ni^{2+}	-diversify by adding As^{5+} → oxyanion-forming, metabolically available metalloid
Bentonite clay smectite content	-metal remobilization observed in presence of low-smectite clays; no remobilization in presence of high smectite clays	-investigate sorption onto three bentonite clays of differing smectite content (15-58%)
Relative geochemical influence of bacterial enrichments	-little change in clay-endemic IRB-containing microcosms relative to sterile controls (viability?); influence of Oil Sands enrichment more prominent	-augment with IRB from Oil Sands and base metal mine tailings environments

PHREEQC Interactive 2.15.0 (version 2.15.0.2697, United States Geological Survey, available for download at http://wwwbrr.cr.usgs.gov/projects/GWC_coupled/phreeqc/) was used to model abiotic geochemical parameters within experimental microcosms for the purpose of approximating Cu^{2+} and Cd^{2+} speciation.

3.5 Dissolved Metal(loid) Quantification

Filtrate samples obtained from batch sorption experiments (see Section 3.4) were analyzed via Inductively Coupled Plasma – Mass Spectrometry (ICP-MS; Perkin Elmer SCIEX ELAN 6100, Woodbridge, ON, Canada) or Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP-OES; Thermo Jarrel Ash IRIS Advantage Spectrophotometer; Franklin, MA, U.S.A.) to determine dissolved concentrations of each analyte of interest (Cu^{2+}, Ni^{2+}, Cd^{2+} for preliminary batch sorption experiments; Cu^{2+}, Cd^{2+}, As^{5+} for principal batch sorption experiments). Samples requiring dilution for ICP-MS analysis were diluted volumetrically in triplicate in a solution of 2% trace metal grade HNO_3, and average analyte concentrations for triplicates are reported herein. Although ICP-OES analysis required no dilution, analyte concentrations were obtained in triplicate and average concentrations are reported herein. Appropriate blanks of 2% HNO_3 and Milli-Q water were analyzed to ensure metal(loid) cleanliness throughout experimental procedures.

Where applicable, data interpretation involved regression analysis to verify statistical significance of results. R^2 values reported in Section 4.2.2 describe the influence of pH on % metal(loid) bound in a given treatment to a given bentonite clay substrate. A sample size of $n = 6$ and, unless otherwise indicated, a significance level of $p < 0.05$ was applied.

3.6 Bacterial Community Composition Analysis

Bacterial community composition of environmental enrichments was determined via 16S rRNA gene sequencing. DNA extraction employed the PowerSoil^{TM} DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA), and was used in accordance with the manufacturer's protocol. DNA was amplified via the polymerase chain reaction (PCR) using primers 27F and 1492R targeting the 16S rRNA gene. Visualization of the PCR products was accomplished via gel electrophoresis through a 1% agarose gel augmented with ethidium bromide, and subsequent illumination under UV light. Desired bands were excised and the purified PCR product recovered (MinElute®, Qiagen Inc., Toronto, ON, Canada) as per the manufacturer's protocol. Purified PCR products were cloned using the pCR^{TM}4-TOPO® vector (TOPO TA cloning kit, Invitrogen Canada Inc., Burlington, ON, Canada). Vectors were heat shocked and transformed into chemically-competent *Escherichia coli* (Top-10), and 100μL aliquots of the *E.coli* suspension were plated onto solid carbenicillin-amended Luria-Bertani (LB) media plates and incubated for 24hours at 37°C. After 24hours, individual colonies were isolated under sterile conditions within the BSC, resuspended in 2mL of carbenicillin-amended LB broth, and incubated at 37°C for an additional 12 hours. Bacterial cells were then separated via centrifugation at 6 800 x g for three minutes, and the pelleted cells were stored at -20°C until required for mini-prepping. Samples were mini-prepped using the QIAprep® Spin Miniprep Kit (Qiagen Inc., Toronto, ON, Canada) as per the manufacturer's protocol, and 2μL of mini-prepped product was added to 2μL M13R primer within 96-well plates. Samples were thereafter sequenced at the McMaster Institute for Molecular Biology and Biotechnology (MOBIX Lab, McMaster University, Hamilton, ON, Canada) using ABI BigDye terminator chemistry (3730 DNA Analyzer, Applied Biosystems, Foster City, CA, U.S.A.). Sequences were evaluated against the nucleotide BLAST (Basic Local Alignment Search Tool; http://blast.ncbi.nlm.nih.gov/Blast.cgi) database software.

3.7 Epifluorescence Microscopy

Live/dead staining (LIVE/DEAD® BacLight™ Bacterial Viability Kit, Invitrogen Canada Inc., Burlington, ON, Canada) was used to qualitatively confirm bacterial viability in environmental enrichments and at the conclusion of batch sorption experiments, and was conducted in accordance with the manufacturer's protocol. This technique utilizes two nucleic acid stains, propidium iodide and SYTO® 9; SYTO® 9 stains bacteria independently of cell viability, while propidium iodide stains only dead cells, requiring sufficient membrane permeability (i.e. damage) to facilitate penetration of the stain into the cell. Thus, this technique allows for the identification of live and dead bacterial cells, such that live cells fluoresce green and dead cells fluoresce red. Epifluorescence microscopy utilized a Leica LEITZ DMRX epifluorescence microscope equipped with an HBO 100-W mercury arc lamp (Leica Microsystems Canada, Richmond Hill, ON, Canada).

3.8 XRD Characterization

Bentonite clay sample mineralogy was determined via X-ray diffraction (XRD) at the McMaster Analytical X-Ray Diffraction Facility (MAX, McMaster University, Hamilton, ON, Canada). XRD spectra were collected for unmodified bentonite clay samples to determine original sample mineralogy; samples were powdered by hand using a mortar and pestle, and the size fraction <100μm was analyzed. To discern mineralogical changes resultant from microbial alteration of the initial solid phase, XRD spectra were also collected for bentonite samples used as sorbents in the principal batch sorption experiments. These samples, retained from the final (six week) sampling period, were dried at 40°C for 16 hours and were powdered. XRD spectra were collected at 25°C using a Bruker AXS D8 Discover Powder X-ray Diffractometer featuring a GADDS detector. Samples were exposed to Cu Kα1 radiation generated at 40mA and 40kV, and scans were conducted at 2θ values from 5 to 65.

4.0 Results and Discussion

4.1 XRD Characterization

Mineralogical examination of the bentonite clay samples utilized in the principal batch sorption experiments was accomplished via XRD. Mineralogy is provided for initial and post-sorption experiment bentonite clay samples in Tables 4.1-4.3, with smectite content highlighted in red. The bentonite clays are mineralogically-distinct, supporting the likelihood of variability with regards to sorptive properties as well as iron bioavailability. Important distinctions between bentonite clay samples are derived from the relative abundance of a number of mineralogical phases; these include smectites, illites, and chlorites, three important clay mineral sorptive phases, calcite and dolomite, carbonate minerals which contribute to the buffering capacity of the bentonite clays, quartz, a relatively inert phase with regards to bacterial nutrient acquisition and metal(loid) sorption, zeolites, important porous sorbent phases, and amorphous material, presumably comprised of organics and/or iron oxyhydroxides, which are important metal(loid) sorbents and sources of Fe^{3+} in the environment.

Table 4.1 – High-smectite bentonite clay sample mineralogy

	Initial	**T1 w As**	**T1 no As**	**T2 w As**	**T2 no As**	**T3 w As**	**T3 no As**
Quartz	**7%**	6%	7%	7%	6%	6%	8%
Calcite	**5%**	4%	3%	3%	2%	3%	4%
Albite/Anorthite	**12%**	13%	13%	12%	13%	12%	12%
Microcline	**2%**	3%	2%	2%	3%	2%	3%
Illite/Mica	**4%**	4%	3%	3%	4%	3%	2%
Chlorite	**2%**	0%	1%	1%	1%	1%	1%
Smectite	**58%**	47%	44%	40%	41%	46%	48%
Cristobalite	**5%**	5%	5%	5%	5%	4%	4%
Halite	**0%**	0%	0%	0%	0%	1%	0%
Amorphous, etc.	**5%**	18%	22%	27%	25%	22%	18%

Table 4.2 – Medium-smectite bentonite clay sample mineralogy

	Initial	**T1 w As**	**T1 no As**	**T2 w As**	**T2 no As**	**T3 w As**	**T3 no As**
Quartz	**20%**	20%	19%	19%	19%	20%	21%
Calcite	**3%**	3%	1%	0%	1%	1%	0%
Albite/Anorthite	**15%**	14%	14%	14%	14%	14%	15%
Microcline	**3%**	3%	4%	3%	3%	3%	3%
Illite/Mica	**15%**	6%	12%	14%	11%	15%	13%
Chlorite	**6%**	4%	4%	3%	4%	3%	5%
Smectite	**29%**	20%	18%	19%	26%	20%	28%
Cristobalite	**4%**	3%	3%	4%	3%	4%	5%
Halite	**0%**	0%	0%	1%	0%	0%	0%
Amorphous, etc.	**5%**	27%	25%	23%	19%	20%	10%

Table 4.3 – Low-smectite bentonite clay sample mineralogy

	Initial	**T1 w As**	**T1 no As**	**T2 w As**	**T2 no As**	**T3 w As**	**T3 no As**
Quartz	**18%**	17%	17%	17%	19%	17%	17%
Calcite	**5%**	0%	0%	0%	0%	0%	2%
Albite/Anorthite	**15%**	14%	14%	16%	16%	16%	14%
Microcline	**2%**	3%	2%	3%	3%	3%	3%
Dolomite/Ankerite	**1%**	0%	1%	1%	0%	0%	1%
Illite/Mica	**20%**	15%	14%	18%	19%	20%	19%
Chlorite	**1%**	0%	0%	1%	0%	2%	2%
Smectite	**15%**	14%	10%	14%	14%	15%	14%
Zeolite	**12%**	4%	3%	3%	3%	3%	3%
Cristobalite	**6%**	5%	5%	5%	5%	5%	5%
Halite	**0%**	1%	0%	0%	0%	0%	0%
Amorphous, etc.	**5%**	27%	34%	22%	21%	19%	20%

4.2 Principal Batch Metal(loid) Experiment Solution Assays

4.2.1 Influence of Solution pH with Time

To discern the influence of Fe-reducing bacterial augmentation on solution pH, this parameter was monitored at each sampling period, and measurements were compared to sterile controls as a baseline. Sterile controls delineate pH change resultant of bentonite clay substrate buffering capacity upon exposure to the metal(loid) solution (initially at pH = 4.00) exclusive of any microbial activity. No chemical buffering agents were added, thus proton changes are reflected in observed pH measurements.

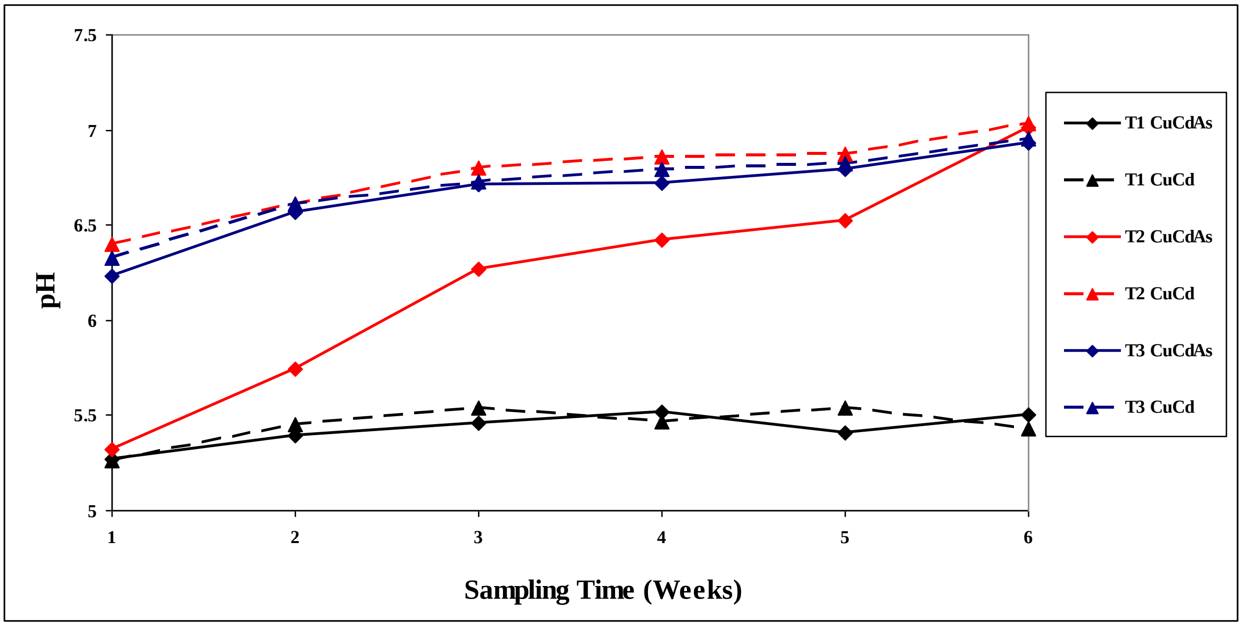


Fig. 4.1 – pH versus sampling time, high-smectite bentonite clay experiments

All graphs herein utilize uniform legend symbols and colours throughout (see legend in Figure 4.1). Note that T1 refers to Treatment 1 (i.e. sterile), T2 refers to Treatment 2 (base metal mine tailings IRB enrichment), and T3 refers to Treatment 3 (Oil Sands composite tailings IRB enrichment). Dashed lines represent non-As-amended treatments, while solid lines represent As-amended treatments.

Figure 4.1 shows solution pH measured in the high-smectite bentonite clay batch sorption experiments against sampling time for experimental treatments. Changes in pH observed in sterile controls represent strictly abiotic mineralogical and solution chemistry effects resultant from the addition of metal(loid)/acetate solution to the bentonite clay sample, and are proposed to comprise a component of the pH change observed in bacterially-augmented treatments as well. However, a clear distinction is observed between pH trends characterizing sterile controls and those describing bacterially-augmented treatments, substantiating the important role that bacterial metabolic processes assume in controlling solution pH.

With the addition of pH = 4.00 metal(loid)/acetate solution to the high-smectite bentonite clay, sterile treatments showed an increase in pH by ~1.25 units, after which, minimal variation in solution pH was observed. The initial increase in pH is attributed to the dissolution of 1-2% calcite present in the clays (see Equation 4.1), as well as several percent smectite and chlorite. In addition to calcite dissolution, the dissolution of clay minerals in acidic solution, namely smectite and chlorite, is known to be proton consuming (Metz et al., 2005, Lowson et al., 2007). Equation 4.2 shows the dissolution of the smectite montmorillonite in acidic solution according to Metz et al. (2005), and Equation 4.3 represents the dissolution of the chlorite ripidolite in acidic solution according to Lowson et al. (2007). These proposed abiotic mechanisms driving pH increase are supported by post-experiment XRD data (see Table 4.1), which confirms a decrease in the relative abundance of calcite, smectite and chlorite mineral phases across treatments.

Eq. 4.1 $CaCO_3 + \mathbf{H^+} \rightarrow Ca^{2+} + \mathbf{HCO_3^-}$

Eq. 4.2 $^*M_{0.33}[Mg_{0.66}Al_{3.34}]^{VI}[Si_8]^{IV}O_{20}(OH)_4\cdot(H_2O)_n + \mathbf{12H^+} + 8H_2O \rightarrow 0.33M^{2+} + 0.66Mg^{2+} + 3.34Al^{3+} + 8H_4SiO_4 + nH_2O$

*M in this formula represents 0.33 divalent exchangeable cations (e.g. Ca^{2+}) or 0.66 exchangeable monovalent cations (e.g. Na^+, K^+)

Eq. 4.3 $(Mg_{2.76}Fe^{2+}_{1.90}Fe^{3+}_{0.07}Al_{0.97})[Si_{2.48}Al_{1.52}O_{10}](OH)_8 + \mathbf{18.08H^+} \rightarrow 2.76Mg^{2+} + 1.90Fe^{2+} + 0.07Fe^{3+} + 2.49Al^{3+} + 2.48H_4SiO_4 + 8.08H_2O$

Further alteration of solution pH across treatments may be derived from the precipitation of amorphous Fe-oxyhydroxides, corresponding to the re-oxidation of Fe^{2+} generated via mineral dissolution and/or Fe^{3+} reduction. Although no confirmatory evidence for explicit oxyhydroxide formation in these experiments exists, a large increase in the relative abundance of a solid phase amorphous component is observed across treatments (see Table 4.1). Presumably, a portion of this material is Fe-oxyhydroxides, the formation of which is acid producing (see Equation 4.4). Proton generation via Fe-oxyhydroxide precipitation would offset a portion of the alkalinity generated via proton-consuming dissolution reactions; however, accurate determination of acid/base chemistry in these experiments was not possible. An inherent limitation of the technique, X-ray diffraction is unable to distinguish among amorphous phases, meaning that amorphous material detected via XRD can be comprised of numerous non-crystalline phases (e.g. Fe-oxyhydroxides, organics, etc.), thereby preventing quantification of Fe-oxyhydroxide relative abundance and hence, detailed acid/base calculations.

Eq. 4.4 $Fe^{3+} + 2H_2O \rightarrow FeOOH + \mathbf{3H^+}$

A number of processes, including those discussed above to apply for sterile controls (i.e. calcite dissolution of 1-3%, clay mineral dissolution by several percent, oxyhydroxide/amorphous formation), as well as metabolic activity (i.e. organic carbon oxidation, Fe(III) reduction), are postulated to influence solution pH in bacterially-augmented treatments. Contrasting results for sterile controls, all bacterially-augmented treatments increased to circumneutral pH within the experimental timeframe. The change in solution pH observed in bacterially-augmented treatments in comparison with sterile controls is postulated to result, in part, from oxidation of the organic carbon source, acetate, coupled to the reduction of molecular oxygen prior to the onset of Fe^{3+} reduction. This process generates bicarbonate alkalinity as per Equation 4.5.

Eq. 4.5 $CH_3COO^- + 2O_2 \rightarrow \mathbf{2HCO_3^-} + \mathbf{H^+}$

Yet another potentially important biologically-mediated process which would influence solution pH as well as composition of the solid and solution phases is derived from the reductive dissolution of Fe-oxyhydroxides, coupled to the oxidation of acetate (see Equation 4.6). Amorphous Fe-oxyhydroxides are widely recognized as an important Fe^{3+} source for Fe-reducing bacteria in the environment, and have been shown to undergo reductive dissolution in the presence of Fe-reducing bacteria in numerous studies to date (e.g. Lovley & Phillips, 1986ab, Caccavo et al., 1996, Cummings et al., 1999, Zachara et al., 2002, Bonneville et al., 2004). Apart from the direct influence on system pH attributed to the consumption of protons and generation of bicarbonate alkalinity via reductive dissolution of amorphous Fe-oxyhydroxides as per Equation 4.6, this process can also influence metal and metalloid dynamics resultant from concurrent remobilization of oxyhydroxide-sorbed metal(loid)s upon dissolution.

Eq. 4.6 $8FeOOH + CH_3COO^- + \mathbf{15H^+} \rightarrow 8Fe^{2+} + \mathbf{2HCO_3^-} + 12H_2O$

Metalloid As-amended and non-amended T3, as well as non-As-amended T2 solutions increase in pH to similar levels by the first sampling period; As-amended T2, however, displays a lag in pH increase across the 1-5 week sampling periods. The reduction of As^{5+} coupled to the oxidation of acetate is a proton consuming reaction (see Equation 4.7); however, in aqueous solution, As^{5+} primarily exists as an oxyanionic species, AsO_4^{3-}. The reduction of the oxyanionic species of As^{5+} coupled to the oxidation of acetate proceeds via a proton-consuming reaction (see Equation 4.8). Thus, the observed relative lag in pH increase in As-amended T2 is likely not explained by the reduction of AsO_4^{3-}, as this reaction leads to the consumption of protons; rather, this lag is more consistent with an inhibition of bacterial activity in the presence of As, a metalloid known to demonstrate toxic effects (e.g. Cervantes et al., 1994, Turpeinen et al., 2004), for instance, via the inhibition of sulfhydryl-bearing enzymes or substrate-level phosphorylation (Kocar et al., 2006), and/or a shift in the predominant metabolically-active bacterial species in favour of more metal(loid)-resistant bacteria (e.g. Kelly et al., 1999, Gremion et al., 2004, Macur et al., 2004). Contrarily, T3 and non-As-amended T2 show an increase in solution pH compared to sterile controls as early on in the experimental time scale as the first sampling period; these results support the interpretation that pH increase in these treatments is related to the bacterial oxidation of acetate coupled to the reduction of oxygen and/or the reductive dissolution of Fe-oxyhydroxides coupled to the oxidation of acetate.

Eq. 4.7 $4As^{5+} + CH_3COO^- + 4H_2O \rightarrow 4As^{3+} + \mathbf{2HCO_3^-} + \mathbf{9H^+}$

Eq. 4.8 $4AsO_4^{3-} + CH_3COO^- + \mathbf{7H^+} \rightarrow 4AsO_2^- + \mathbf{2HCO_3^-} + 4H_2O$

Augmentation of the high-smectite bentonite clay treatment solutions with Fe-reducing bacterial enrichments was shown to influence solution pH throughout the duration of these experiments (see Figure 4.1). Recalling the importance of pH on metal(loid) mobility in the environment (Hill, 1997), the observed pH trends illustrate an important facet of the potential bacterial influence on metal(loid) dynamics, sorptive behaviour, and cycling in aqueous solution.

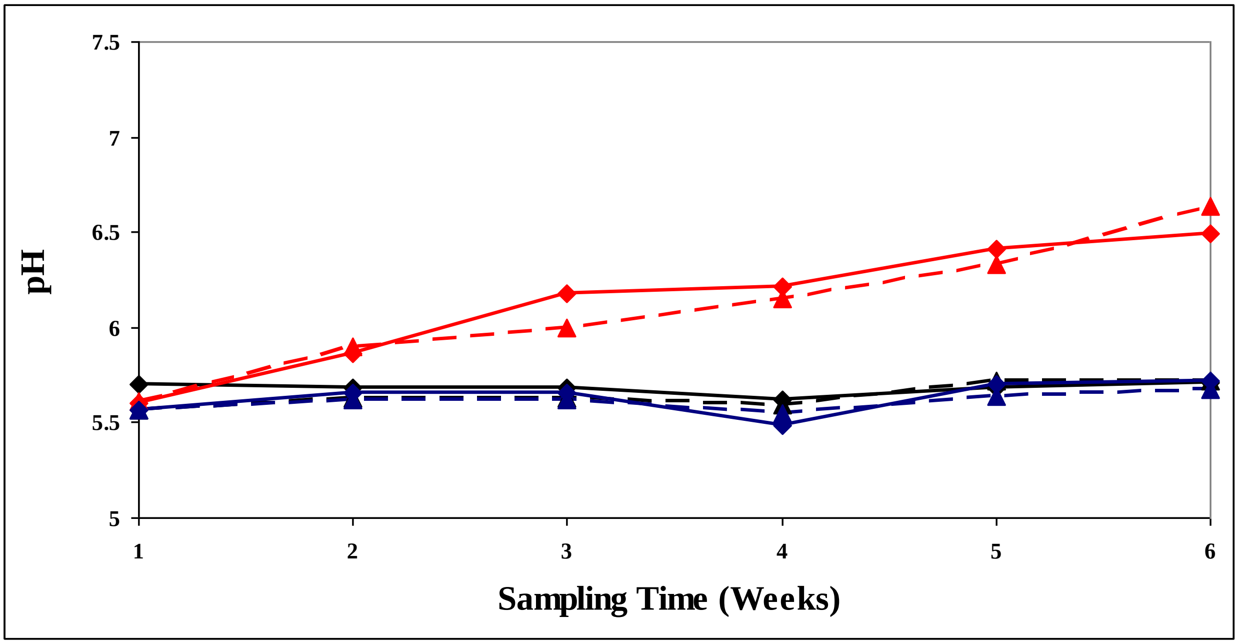


Fig. 4.2 – pH versus sampling time, medium-smectite bentonite clay experiments

Figure 4.2 shows solution pH versus sampling time in batch sorption experiments employing bentonite clay of medium smectite content. Sterile controls (T1) show minimal variation in pH throughout the duration of the sampling campaign after an initial increase by ~1.5 units, and this trend is largely matched by trends observed in T3. T2, regardless of As augmentation, shows increasing solution pH over time to a maximum of 6.49 (As-amended) and 6.64 (non-amended).

XRD analysis (see Table 4.2) of residual solid phase material from T1 confirmed the dissolution of 0-1% calcite, 9-11% smectite, 2% chlorite, and 3-9% illite (see Equation 4.9, according to Köhler et al., 2003 and references therein). pH trends observed in T1 and T3 are similar to those observed for sterile controls in the high-smectite bentonite clay experiments described above, i.e. an initial increase in solution pH with the addition of bentonite clay sample to metal(loid)/acetate solution at pH = 4.00, corresponding to the proton-consuming dissolution of various mineral phases (see Equations 4.1-4.3, 4.6 and 4.9), followed by negligible change in pH thereafter. T3 pH trends strongly resemble those observed in T1, and are attributed to a decrease in the relative abundance of calcite by 2-3%, illite by 0-2%, chlorite by 1-3%, and smectite by 1-9% (see Table 4.2). Similarities in pH trends observed in T1 and T3 propose the cessation of microbial metabolic activity in T3, and this is largely supported by similarities in metal(loid) sorptive behaviour between T1 and T3 (see Section 4.2.2). The presumed cessation of microbial metabolic activity observed in T3 is the sole example of such an occurrence in all experimental treatments across clay samples evaluated. This finding is postulated to indicate the existence of a bentonite clay-based control which dictates observed metal(loid) toxicity and/or increases the extent of bacterial exposure to the toxic elements. Indeed, attempts to re-culture bacteria from residual slurry sample at the conclusion of these experiments yielded two interesting findings: a) bacterial viability was confirmed, but a ~three week lag period was required between inoculation and visible onset of turbidity, perhaps indicating bacterial dormancy; and b) Fe^{3+}-reduction was not achieved by bacteria from the As-augmented T3 slurry when re-suspended in fresh media, supporting that bacteria responsible for iron reduction in the Oil Sands composite tailings enrichment encountered toxicological inhibition (see Section 4.3.2). Despite bacterial viability confirmation upon re-suspension in fresh media, microbial metabolic activity was likely inhibited in the T3 medium-smectite bentonite clay experiments, leading to the observed similarities in solution chemistry (namely, pH trends and metal(loid) behaviour) characterizing T1 and T3.

Eq. 4.9 $(Si_{3.55}Al_{0.45})(Al_{1.27}Fe^{(III)}_{0.36}Mg_{0.44})O_{10}(OH)_2(Ca_{0.01}Na_{0.13}K_{0.53}) + \mathbf{7.80H^+} \rightarrow 3.55SiO_2 + 1.72Al^{3+} + 0.36Fe^{3+} + 0.44Mg^{2+} + 0.01Ca^{2+} + 0.13Na^+ + 0.53K^+ + 4.90H_2O$

Trends observed in T2, in comparison to those characterizing T1 and T3, display an increase in pH over time, and show similarities to trends observed in As-amended T2 of the high-smectite bentonite clay experiments. The proton-consuming mineral dissolution processes (see Equations 4.1-4.3, 4.6, and 4.9) described above to influence T1 and T3 undoubtedly influenced solution pH in T2, and XRD confirmed a decline in calcite relative abundance by 2-3%, illite by 1-4%, chlorite by 2-3%, and smectite by 3-10%. In addition, the aerobic oxidation of acetate (as per Equation 4.5), reductive dissolution of Fe-oxyhydroxides (as per Equation 4.6), and potential reduction of arsenate (Equation 4.8), are postulated to result in increased solution pH in T2 solutions relative to sterile controls.

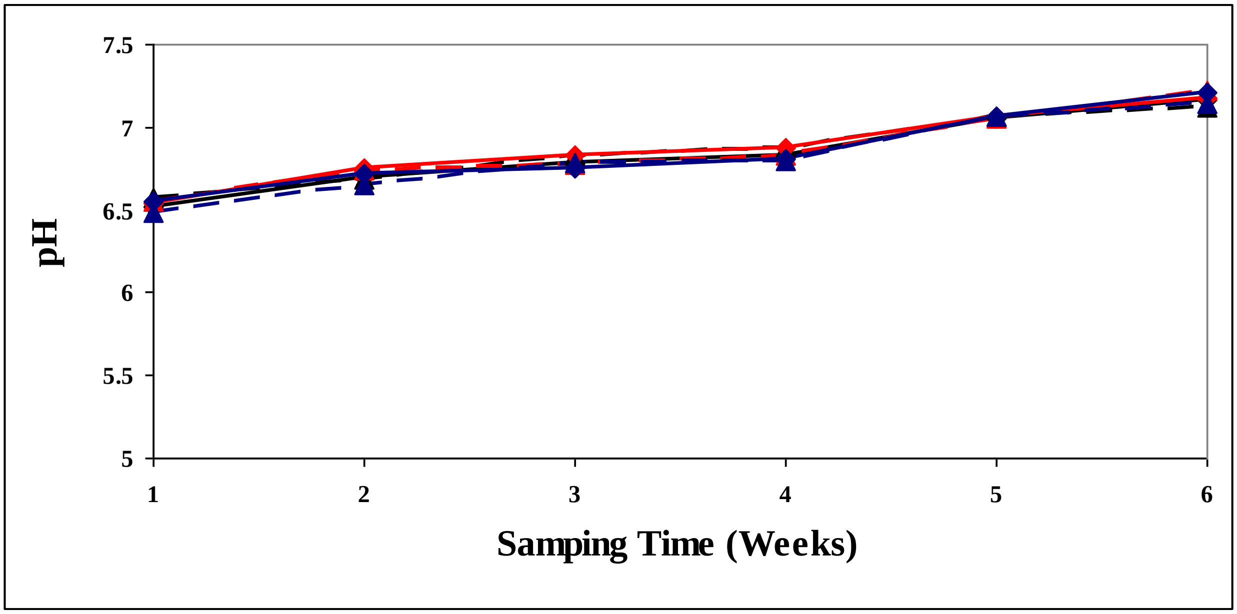


Fig. 4.3 – pH versus sampling time, low-smectite bentonite clay experiments

Figure 4.3 shows solution pH measured in the low-smectite bentonite clay batch sorption experiments against sampling time for bacterially-augmented and sterile control treatments. Observed pH trends are very similar for all treatments throughout the duration of these experiments. Notably, the minimal differences existing between the pH of sterile and microbial treatments suggests that augmentation with Fe-reducing bacterial enrichments showed negligible influence on solution pH in the presence of the low-smectite bentonite clay. Upon reaching the one week sampling period, it is recognizable that the addition of bentonite clay to the metal(loid)/acetate solution raised the pH of all treatments from 4.0 to roughly 6.5. This result is explained by the greater relative abundance of carbonate minerals in the low-smectite bentonite clay and correspondingly higher buffering capacity of this clay sample in comparison with the high- and medium-smectite bentonite clays. Proportionally, the low-smectite bentonite clay contained the greatest abundance of carbonate minerals of the three clays evaluated in the principal batch sorption experiments, and was comprised of 5% calcite and 1% dolomite (see Table 4.3).

Calcite dissolution (see Equation 4.1) proceeded to completion in all treatments with the exception of T3 non-As amended, which contained 2% calcite at the conclusion of the experiments. Similarly, dolomite dissolution (see Equation 4.10) was observed in a number of treatments, namely T1 As-amended, T2 non-As amended, and T3 As-amended. Illite dissolution proceeded to 0-6%, providing buffering capacity according to Equation 4.9. The circumneutral pH conditions resultant of calcite, dolomite and illite dissolution are unfavourable towards the proton-consuming dissolution of smectite, and this is reflected by the minimal decline observed in smectite relative abundance across treatments (typically 0-1% decrease, with one exception reaching 5%). As observed in the high- and medium-smectite bentonite clay experiments, a large increase in relative abundance of amorphous material is also detected in low-smectite bentonite clay residual solid phase, supporting the potential influence of Fe-oxyhydroxide precipitation and/or dissolution reactions on solution pH (see Equation 4.4 and 4.6).

Eq. 4.10 $CaMg(CO_3)_2 + \mathbf{2H^+} \rightarrow Ca^{2+} + Mg^{2+} + \mathbf{2HCO_3^-}$

Despite the apparent lack of influence of T2 and T3 bacterial augmentation on solution pH in the presence of the low-smectite bentonite clay, perhaps superficially suggesting non-viability of augmented bacteria, examination of As metalloid dynamics in particular (Section 4.2.2.3) suggest that bacterial viability was indeed observed and further, assumed an important role in controlling metalloid sorption in these experiments. While fluctuations in pH are often regarded as a proxy for microbial metabolic activity, observations made in the low-smectite bentonite clay experiments represent an important deviation from this assumption, supporting that negligible change in pH in the presence of microorganisms does not necessarily indicate a lack of microbial metabolic activity. Although geochemical confirmation of bacterial viability in T2 and T3 via observation of As sorption/desorption dynamics is not possible in the non-As amended treatment, results presented in Section 4.2.2.3 indicate that microbial activity will not always result in observable pH differences relative to sterile controls. Indeed, all microbial treatments from the low-smectite bentonite clay experiments were shown to contain viable bacteria at the conclusion of these experiments, and further, all bacterial treatments were observed to reduce Fe^{3+} when suspended in fresh media at the end of the experiments (see Section 4.3.2). Hence, despite an apparent lack of any difference in solution pH observed in T2 and T3 of the low-smectite bentonite clay experiments compared to sterile controls, metalloid dynamics and re-growth attempts at the conclusion of the principal batch metal(loid) sorption experiments indicate that bacterial viability persisted in these treatments.

The buffering capacity demonstrated by this particular bentonite clay sample was sufficient to negate any microbial influence on solution pH, as no apparent distinctions are observed between the pH characterizing microbial treatments from sterile. Importantly, studies have shown that higher carbonate content of sorbent materials has implications for metal mobility, with greater carbonate content and inherently greater buffering capacity facilitating enhanced metal uptake via sorption and precipitation reactions (e.g. Sipos et al., 2009).

Summary and Further Insights:

In summary, these results demonstrate the importance of a number of *interconnected* processes that are predicted to govern solution pH in principal batch metal(loid) sorption experiments, including abiotic mineral and amorphous phase dissolution (e.g. calcite, dolomite, smectite, chlorite, illite, amorphous Fe-oxyhydroxides), and precipitation of amorphous phase material (e.g. Fe-oxyhydroxides), as well as microbially-driven redox reactions involving solution- and solid-phase species (e.g. aerobic oxidation of acetate, reduction of arsenate coupled to the oxidation of acetate, reductive dissolution of amorphous Fe-oxyhydroxides coupled to the oxidation of acetate). As evidenced by pH differences in sterile controls versus select bacterially-augmented treatments, many of these processes were enhanced or facilitated by the activity of Fe-reducing bacterial enrichments, demonstrating the importance of bacterially-driven processes in controlling solid- and solution-phase chemistry.

Several important results concerning the pH of batch metal(loid) sorption experimental solutions in the presence and absence of Fe-reducing bacterial enrichments were identified; among these are: a) that the overall influence of Fe-reducing bacteria on solution pH varies depending on the bentonite clay substrate mineralogy (namely, the presence of carbonate minerals which generate alkalinity, and, when found in abundance, can effectively overshadow the influence of microbial metabolic processes on solution pH as seen herein); b) that an absence of microbially-driven solution pH variation is *not* necessarily indicative of a lack of microbial metabolic activity (i.e. proton generation and consuming processes may be simultaneously occurring); and c) that solution pH in batch sorption experiments, as influenced by substrate acid neutralization capacity and microbial metabolic activity, assumes an important role in controlling metal(loid) sorption and long-term stability of sorbed metals and metalloids *when considering sorption to a single bentonite clay sorbent phase* (see section 4.2.2).

4.2.2 Metal(loid) Sorptive Behaviour

Metal(loid) sorption experiment results are plotted herein as percent metal(loid) bound versus pH; note that, because all microcosms were provided with 1.25g bentonite clay and 25mL sorbate solution at the initiation of these experiments, all results are effectively normalized per gram of sorbent and volume of solution. With these considerations taken into account, plots of percent bound versus pH display relative metal(loid) uptake by a given bentonite clay substrate.

4.2.2.1 Cu^{2+} Sorption

Cu^{2+} was immobilized to near completion (>98.8% sorbed) in all treatments in the presence of the three bentonite clay samples evaluated, showing no significant remobilization throughout the duration of these experiments in any treatment (see Figures 4.4-4.6). Near complete uptake of copper in these experiments is explained by the combined influence of: a) the sorption-related properties of Cu^{2+}; and b) the prevailing pH range characterizing experimental treatments, attributed to the bentonite clay mineralogy and microbial metabolic activity (see Section 4.2.1).

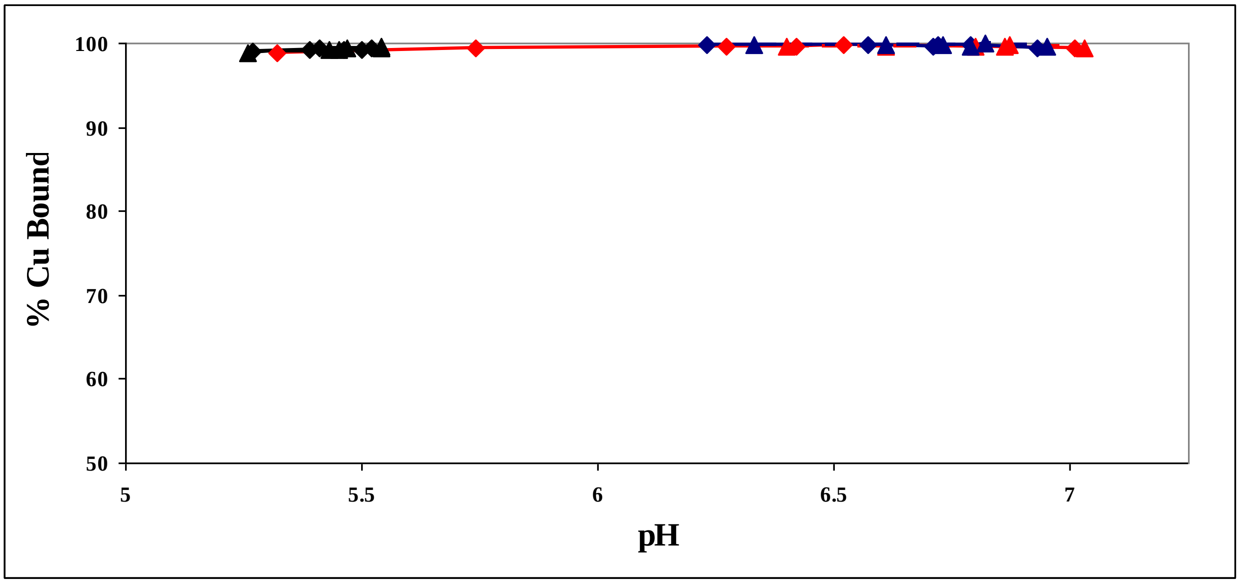


Fig. 4.4 – Bound Cu versus pH, high-smectite bentonite clay experiments

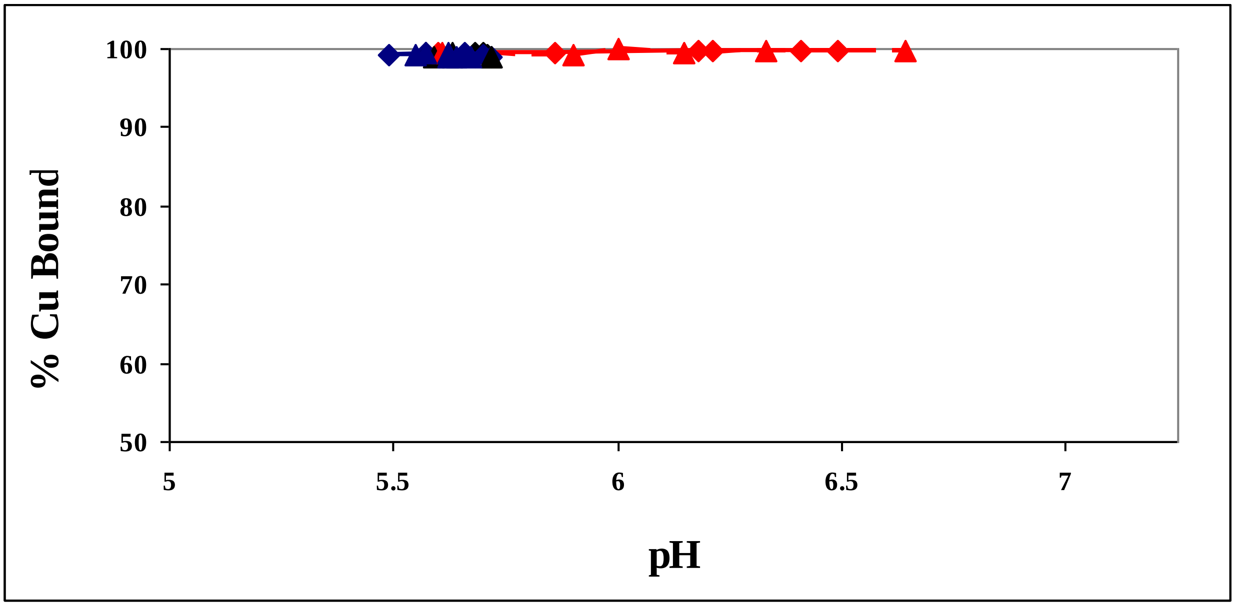


Fig. 4.5 – Bound Cu versus pH, medium-smectite bentonite clay experiments

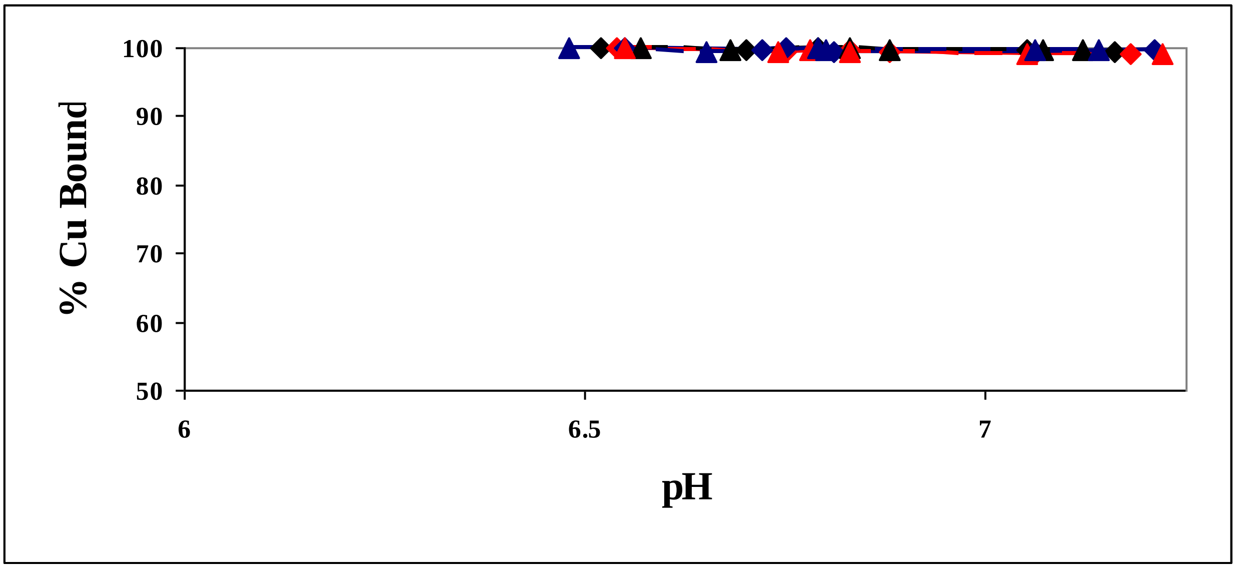


Fig. 4.6 – Bound Cu versus pH, low-smectite bentonite clay experiments

The aqueous Cu^{2+} ion exists as a strong complexing agent, demonstrating a tendency to hydrolyze in aqueous solution to yield the hydrated ionic form, $Cu(H_2O)_6^{2+}$ (Flemming & Trevors, 1989 and references therein). Hydrolysis and precipitation reactions are thus regarded among the greatest determinants of Cu^{2+} chemistry within the pH regime typically characterizing natural waters (Sylva, 1976). In numerous studies to date, various sorbents, including oxidized active carbon (Strelko & Malik, 2002, Strelko et al., 2004), kaolinite (Morley & Gadd, 1995, Yavuz et al., 2003), modified kaolinite (Suraj et al., 1998), montmorillonite (Morley & Gadd, 1995), modified bentonite clay (Olu-Owolabi et al., 2010), fungal/clay mineral composites (Morley & Gadd, 1995), and various soil constituents (Lair et al., 2007, Sipos et al., 2009), have shown specificity towards the uptake of Cu^{2+} in the presence of other divalent cationic metals. This phenomenon is regarded by some authors (e.g. Morley & Gadd, 1995, Strelko & Malik, 2002, Strelko et al., 2004) to occur in accordance with the Irving-Williams series (Irving & Williams, 1953), an order relating transition metal complex stability to the electronic structure of the central metal cation. Sorbent selectivity for Cu(II) is explained by Strelko & Malik (2002) and Strelko et al. (2004) in this context to arise from the enhanced stability of resultant Cu^{2+} complexes, which typically display Jahn-Teller distortion. Jahn-Teller distortion is a tetragonal distortion in which constituent metal-ligand bonds are not of uniform strength, and is derived from the formation of four shorter bonds and two longer trans-bonds resultant of the $3d^9$ electron configuration of Cu^{2+} (Schosseler et al., 1999 and references therein, Strelko & Malik, 2002, Strelko et al., 2004 and references therein, Madden et al., 2006, Persson, 2010). When water molecules associated with a hydrated Cu^{2+} cation are replaced by ligands associated with a sorbent surface, these ligands are proposed to form shorter and inherently stronger metal-ligand bonds, thereby accommodating for increased complex stability (Strelko & Malik, 2002, Strelko et al., 2004 and references therein), and hence, enhanced sorption.

While results of some studies (e.g. Kugler et al., 2002, Kubilay et al., 2007) have identified sorptive selectivity of other cationic metals over Cu^{2+}, a predominantly recurring trend of Cu^{2+} sorptive specificity is clearly observed in the literature. Thus, the near complete extent of Cu^{2+} immobilization observed independently of treatment or bentonite clay substrate in these experiments is not surprising, noting the commonly reported occurrence of copper sorption specificity resulting from the Jahn-Teller distortion displayed by Cu^{2+} complexes. Indeed, an EXAFS spectroscopic investigation of Cu^{2+} sorption to clay sediments by Sajidu et al. (2008) observed the Jahn-Teller distortion in sorbed Cu^{2+} complexes, thereby highlighting the importance of this distortion in controlling Cu^{2+} uptake to clays.

Additionally, the time-dependent increase in solution pH observed in select treatments (i.e. T2 and T3 high-smectite bentonite clay, T2 medium-smectite bentonite clay, and T1-T3 low-smectite bentonite clay; see Figures 4.1-4.3) supports that sorbed Cu^{2+} remobilization is not expected to occur in these treatments, due to the increasing stability of copper precipitate phases (e.g. $Cu(OH)_2$, $Cu_2(OH)_2CO_3$, $Cu_3(OH)_2(CO_3)_2$, etc.; see Sylva, 1976) over this pH regime. Strengthening this rationale, the precipitation of Cu-hydroxides at elevated pH (typically pH > 6.0) is discussed by several authors (e.g. Bhattacharyya & Gupta, 2007, Akar et al., 2009, Eloussaief et al., 2009; reviewed in Bhattacharyya & Gupta, 2008a) to result in Cu^{2+} immobilization, and thus requires consideration in the context of sorption experiments. Copper speciation in experimental microcosms herein is predicted by PHREEQC modelling of abiotic geochemical conditions to be comprised predominantly of Cu^{2+}, $CuHCO_3^+$, $CuCO_3$, $CuOH^+$, $CuCl^+$, and $Cu(OH)_2$, with increasing stability of precipitate phases (i.e. $CuCO_3$ and $Cu(OH)_2$) at elevated pH from 5.0.

In this study, results of XRD mineralogical analysis confirmed a decrease in the relative abundance of calcite and an increase in amorphous content when comparing initial bentonite clay sample compositions with solid phase residual material remaining at the conclusion of these experiments (see Section 4.1). Consistent Cu^{2+} immobilization across treatments and bentonite clay substrates is therefore further explained by the sorption of copper onto the increased-abundance amorphous phase (relying on the highly-plausible assumption that a portion of this phase is an effective sorbent, such as Fe-oxyhydroxides (e.g. Davis & Leckie, 1978, Koeppenkastrop & Decarlo, 1993, Bradl, 2004) and/or organic material (e.g. Kinniburgh et al., 1999, Martinez-Villegas & Martinez, 2008, Plach et al., 2011)), and/or the precipitation of copper hydroxides, carbonates, and hydroxy-carbonates resultant from calcite dissolution coupled to increasing solution pH.

Summary and Further Insights:

In summary, Cu^{2+} sorption to bentonite clay substrates has been shown in these experiments to proceed to near completion in the presence of three mineralogically-distinct bentonite clay sorbents, with negligible remobilization observed across treatments. Cu^{2+} sorption to bentonite clays was demonstrated to occur *independently* of the presence of Fe-reducing bacterial enrichments and As^{5+} augmentation under the experimental conditions detailed herein, and is explained to result from: a) the relative strength of Cu^{2+}-sorption complexes corresponding to the Jahn-Teller distortion; and b) the pH regime characterizing experimental solutions, attributed to the buffering capacity of bentonite clay sorbents, as well as metabolically-coupled acidity consumption and alkalinity generation. Investigation of Cu^{2+} sorption in the presence of Fe-reducing bacterial enrichments under different geochemical conditions than those employed in this thesis may yield additional insights into the sorptive behaviour of copper onto natural bentonite clay sorbents, as well as potential sensitivity to the presence of Fe-reducing bacteria. For instance, conduction of sorption experiments under lower initial solution pH may show time-dependent uptake of Cu^{2+} corresponding to increasing solution pH resultant of microbial metabolic activity; this, however, remains a testable hypothesis. Other important insights may be gained from experimentation utilizing higher initial Cu^{2+} loading or a lower sorbent/sorbate solution ratio.

4.2.2.2 Cd^{2+} Sorption

Cd speciation predicted to exist over the pH range characterizing these experiments is primarily Cd^{2+}, $CdCl^{+}$, $CdHCO_3^{+}$, and lesser amounts of $Cd(OH)^{+}$ (based on PHREEQC modeling of abiotic geochemical parameters, and supported by data in the literature, e.g. Hunter et al., 1999, Lai et al., 2002, Zhao et al., 2011). Unlike Cu^{2+} (see Section 4.2.2.1), loss of Cd^{2+} from solution phase via precipitation is not expected to occur over the pH regime observed in these experiments, and indeed, previous studies have investigated Cd^{2+} sorption onto various substrates, including kaolinite (Gupta & Bhattacharyya, 2008), montmorillonite (Bhattacharyya & Gupta, 2007, Gupta & Bhattacharyya, 2008), and acid-activated montmorillonite (Bhattacharyya & Gupta, 2007) at solution pH up to 10.0, and perlite at pH <9.0 (Mathialagan & Viraraghavan, 2002), without observing metal loss due to precipitation.

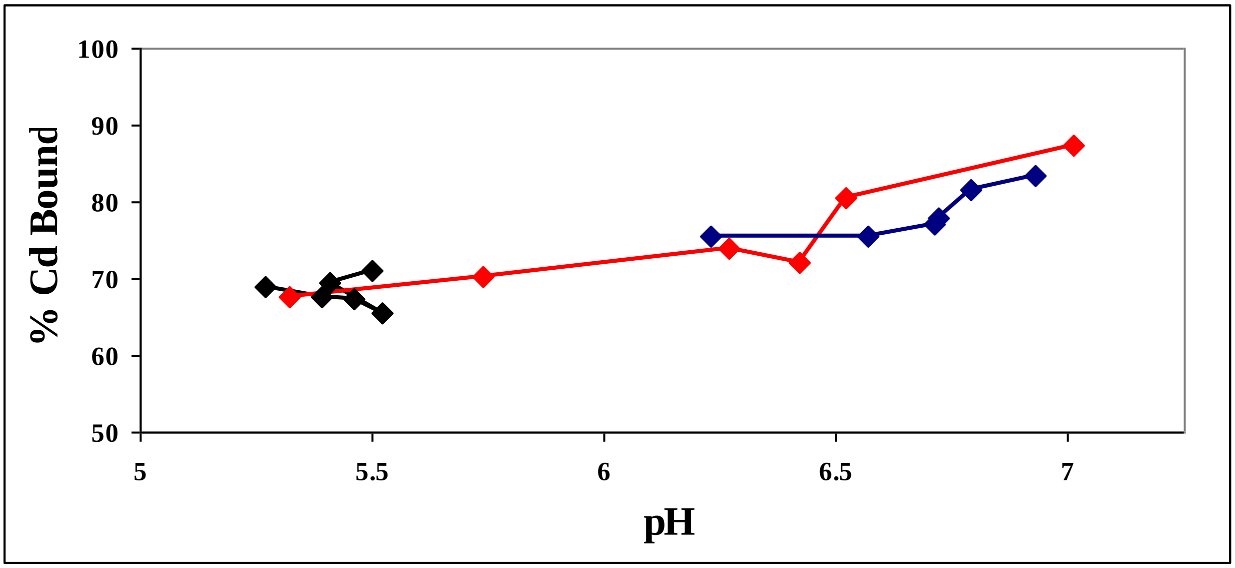


Fig. 4.7 – Bound Cd versus pH (As present), high-smectite bentonite clay experiments

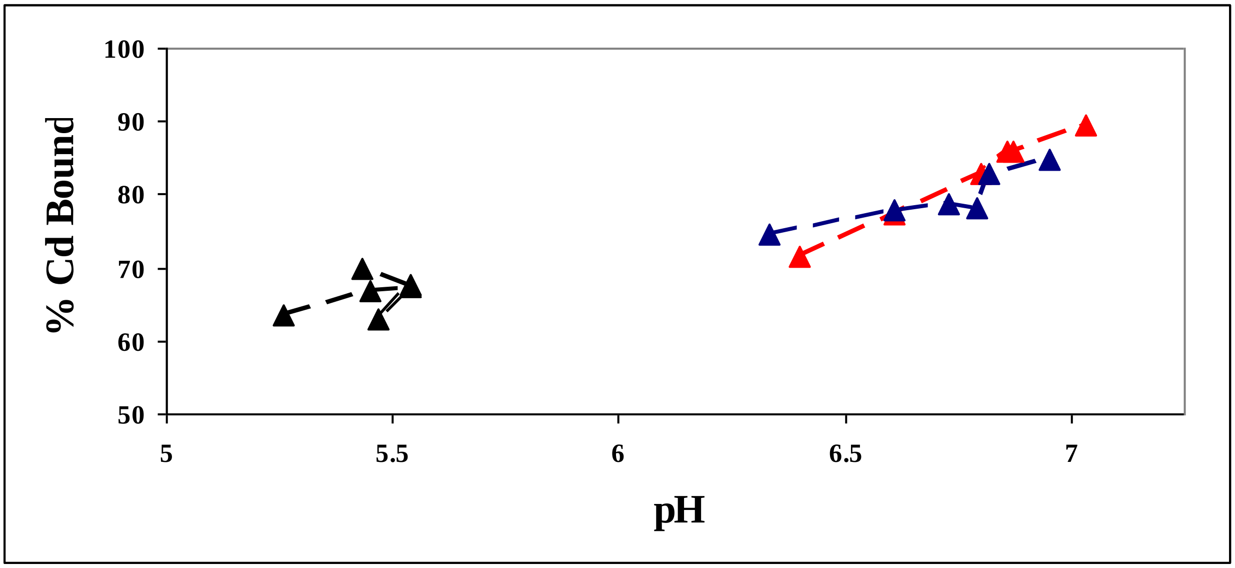


Fig. 4.8 – Bound Cd versus pH (As absent), high-smectite bentonite clay experiments

Cd^{2+} sorption to the high-smectite bentonite clay in the presence and absence of As is shown in Figures 4.7 and 4.8, respectively. T1 sterile controls show similar extents of Cd^{2+} immobilization with no apparent dependency on As augmentation, such that bound Cd^{2+} reaches a maximum of ~70% and ~71% in the absence and presence of As, respectively. The slight increase in % Cd^{2+} bound in T1 over the experimental timeframe, despite the minimal pH variation observed, is attributed to the increased abundance of amorphous material identified via XRD (see Table 4.1) to exist at the conclusion of sorption experiments, inherently providing additional surfaces on which sorption reactions can proceed.

A number of important distinctions between Cd^{2+} sorption trends in T1 versus bacterially-augmented treatments are observed in the presence of the high-smectite bentonite clay. Importantly, T2 and T3 immobilized greater percentages of Cd^{2+} than T1, sorbing ~87%, ~89%, ~83% and ~85% (T2 with and without As, T3 with and without As, respectively) of initial Cd^{2+} loading upon reaching the final sampling period. Overall, sorption of Cd^{2+} in bacterially-augmented treatments displays strong correlation to solution pH (although slightly weaker correlation is observed in As-augmented T3), and can thus be primarily attributed to the increased solution pH characterizing bacterially-augmented treatments (see Section 4.2.1). R^2 values for % Cd^{2+} bound to the high-smectite bentonite clay as a function of solution pH are 0.80 (T2 with As), 0.99 (T2 without As), 0.64 (T3 with As; $p > 0.05$), and 0.81 (T3 without As); in particular, R^2 values for T2 with and without As, and T3 without As show that the majority of variation observed in % Cd^{2+} bound can be explained by variation in pH.

Although As-amended and non-amended T2 solutions both increase in pH to ~7.0 and sorb very similar % Cd^{2+} by the final sampling period, As-amendment induces a lag in the rate of pH increase comparatively (see Section 4.2.1 for interpretation of pH trends). Thus, the importance of pH on Cd^{2+} sorption to the high-smectite bentonite clay as proposed above is well supported by the observed differences between Cd^{2+} sorptive behaviour in T2 in the presence and absence of As. The lagged pH increase observed with As^{5+} amendment correspondingly induces a lag in Cd^{2+} sorption; in the absence of As^{5+}, however, T2 solution pH increases more rapidly, thereby inducing faster uptake of Cd^{2+} from solution. Inhibition of the rate of Cd^{2+} sorption resultant of As^{5+} augmentation is not observed in T3, and is explained by the more rapid increase in pH observed in T3 solutions, despite the presence of As^{5+}. Importantly, the observed disparity between the influence of As^{5+} augmentation on Cd^{2+} sorption in T2 and T3 further supports the pH-dependency of Cd^{2+} uptake onto this specific bentonite clay sorbent; the fact that no influence of As^{5+} augmentation is observed in T3 suggests that the presence of As in solution does not, in itself, affect the sorptive behaviour of Cd^{2+} (for instance, via competitive sorption), with the exception of potential pH influences, such as that observed in T2.

Despite the dissolution of clay mineral sorbent phases evidenced via XRD (see Table 4.1), Cd^{2+} sorption is enhanced in bacterially-augmented treatments due to increased: a) sorptive affinity of the high-smectite bentonite clay for Cd^{2+} with increasing solution pH; and b) abundance of an amorphous phase (presumably comprised of Fe-oxyhydroxides and organics, both of which are effective scavengers of cationic metals; Davis & Leckie, 1978, Koeppenkastrop & Decarlo, 1993, Kinniburgh et al., 1999, Bradl, 2004, Martinez-Villegas & Martinez, 2008, Plach et al., 2011).

The pH-dependency of cationic metal sorption is explained by the increasingly-protonated character of sorbent surface sites under conditions of decreasing solution pH; the abundance of protons existing in solution under acidic conditions effectively out-compete cationic metals such as Cd^{2+} for sorption sites, thereby decreasing cationic metal sorption under low-pH conditions (e.g. Bhattacharyya & Gupta, 2007). Under increasingly alkaline conditions, competition with H^+ for surface sites is diminished, thereby inherently increasing the abundance of surface sites available for cationic metal sorption (Bhattacharyya & Gupta, 2007). Enhanced Cd^{2+} sorption under conditions of increasing solution pH is therefore reported in numerous studies investigating many unique sorbent materials, including Al-, Fe-, and Si-oxide sediment composites (Bradl, 2004 and references therein), perlite (Mathialagan & Viraraghavan, 2002), kaolinite (Gupta & Bhattacharyya, 2008), montmorillonite (Bhattacharyya & Gupta, 2007, Abollino et al., 2008, Gupta & Bhattacharyya, 2008), acid-activated montmorillonite (Bhattacharyya & Gupta, 2007), vermiculite (Abollino et al., 2008), and kaolin (Chantawong et al., 2003), further strengthening the importance of pH on Cd^{2+} sorption to the high-smectite bentonite clay. Interestingly, Cd^{2+} sorption to the high-smectite bentonite clay proceeded to a lesser extent than observed in the low-smectite bentonite clay experiments, coinciding with the lower final solution pH characterizing the high-smectite experiments.

% Cd^{2+} bound to the medium-smectite bentonite clay is shown in Figures 4.9 and 4.10 in the presence and absence of As, respectively. Cd^{2+} behaves very similarly in T1 and T3 regardless of As^{5+} augmentation. Observed uniformities in Cd^{2+} behaviour between the two treatments is attributed to similarities in solution pH (see Section 4.2.1).

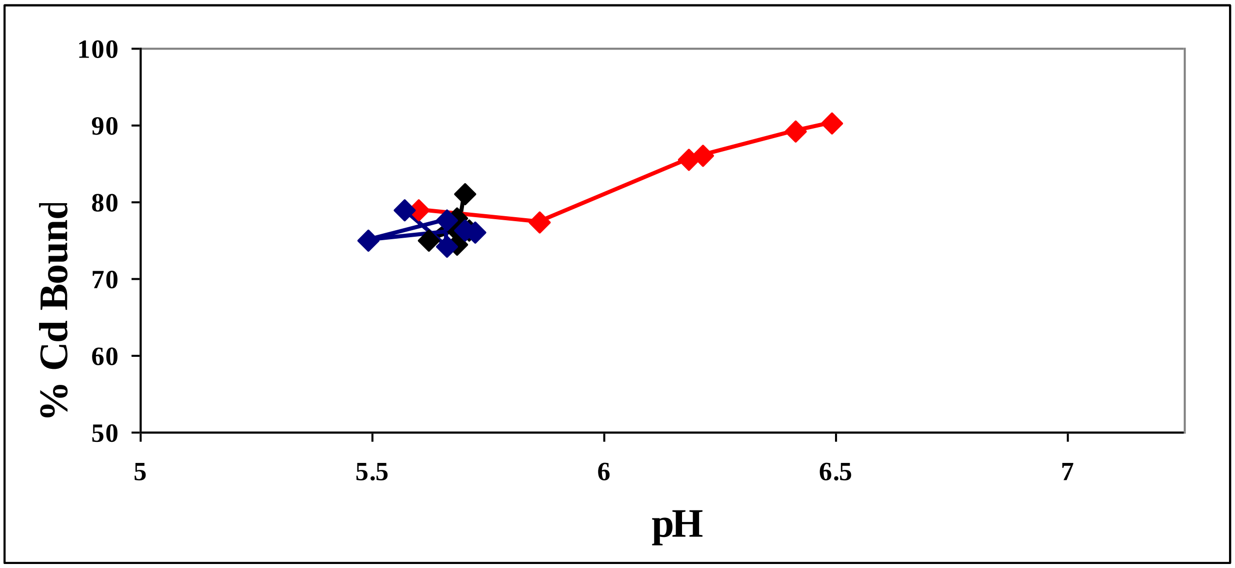


Fig. 4.9 – Bound Cd versus pH (As present), medium-smectite bentonite clay experiments

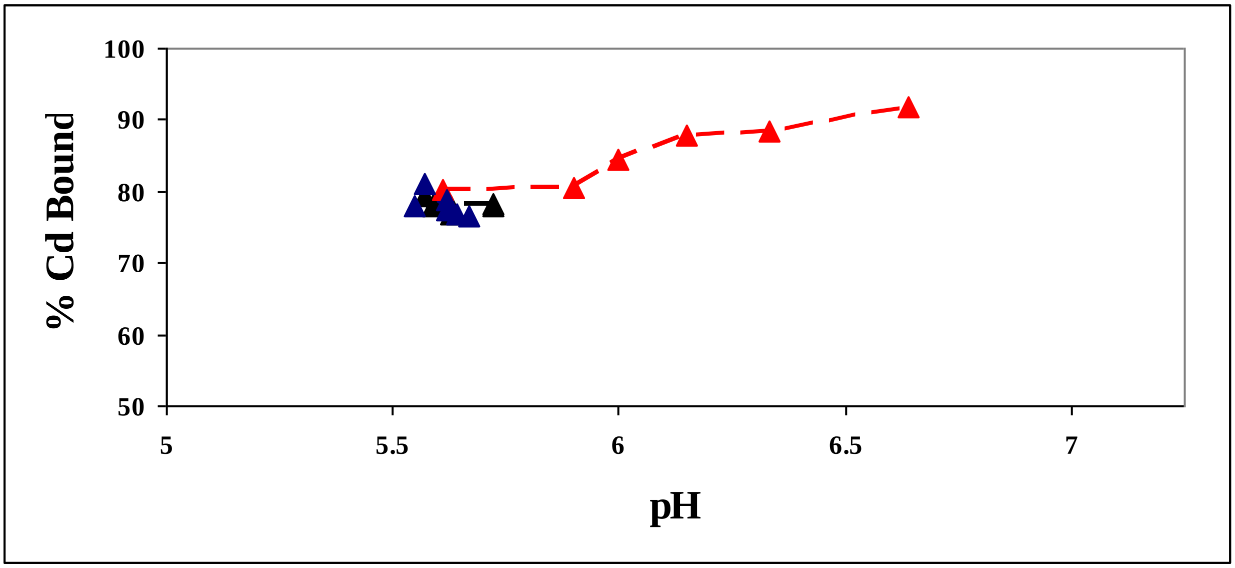


Fig. 4.10 – Bound Cd versus pH (As absent), medium-smectite bentonite clay experiments

Cd^{2+} sorptive behaviour in T2 differs from that characterizing T1 and T3; an increase in bound Cd^{2+} is observed with time, further corresponding to enhanced sorption under conditions of increasing solution pH. This behaviour is observed regardless of As^{5+} augmentation, and trends of % Cd^{2+} bound as a function of pH are described by R^2 values of 0.89 (As^{5+}-amended) and 0.91 (non-amended). These results substantiate the importance of pH on cationic metal sorption to the medium-smectite bentonite clay substrate. However, they further confirm that microbial activity plays an important role driving these reactions through proton balance effects associated with metabolic processes.

% Cd^{2+} bound to the low-smectite bentonite clay in the presence and absence of As is shown in Figures 4.11 and 4.12, respectively.

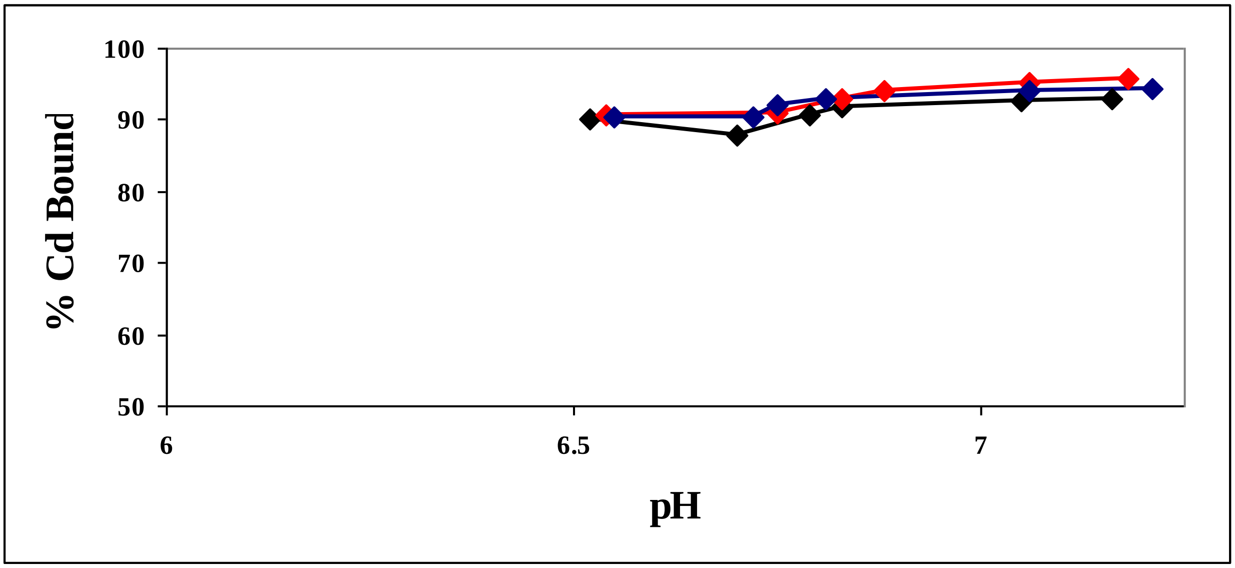


Fig. 4.11 – Bound Cd versus pH (As present), low-smectite bentonite clay experiments

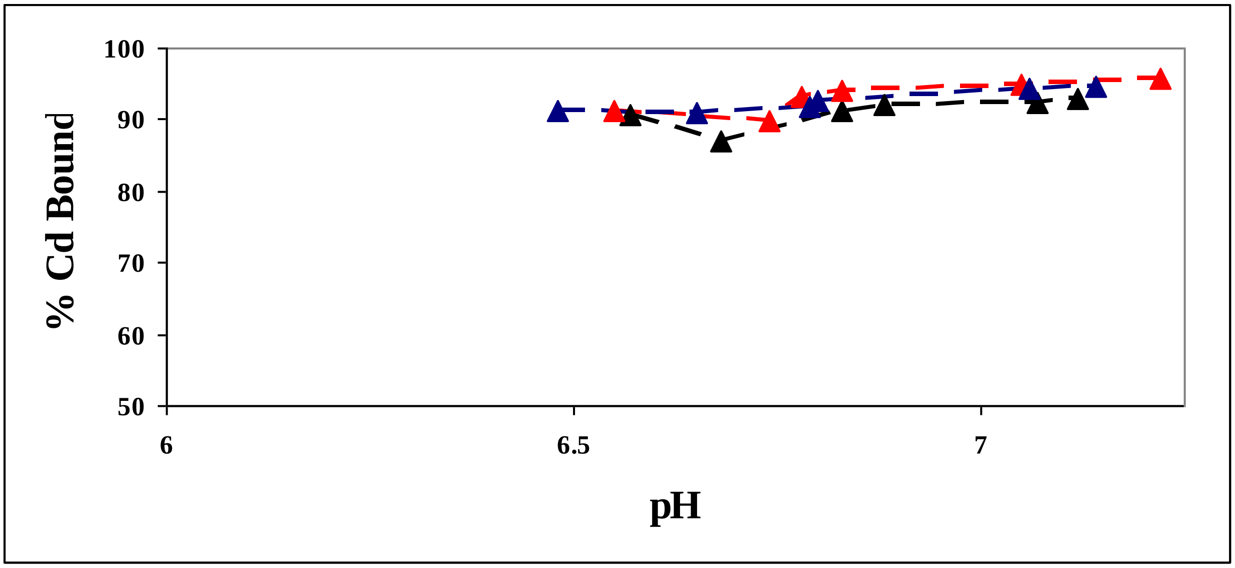


Fig. 4.12 – Bound Cd versus pH (As absent), low-smectite bentonite clay experiments

Minimal differences are observed when augmented with As^{5+}, and all treatments follow the same general trend. Microbially-augmented treatments typically bind Cd^{2+} in slight excess (i.e. <3% more Cd^{2+} bound) when compared to sterile controls at roughly the same pH, perhaps resulting from sorption onto microbial biomass (i.e. biosorption; see, for instance, Volesky, 1987, Lovley & Coates, 1997, Gupta et al., 2000, Barkay & Schaefer, 2001, Lloyd & Lovley, 2001, Valls & de Lorenzo, 2002, Gadd, 2004, Kosolapov et al., 2004, Malik, 2004, Gadd, 2009). However, Cd^{2+} sorption onto the low-smectite bentonite clay in the presence of Fe-reducing bacterial enrichments predominantly reflects abiotic mineralogical and solution chemical controls, as demonstrated by the similarities in Cd^{2+} sorptive behaviour observed across treatments. Resultant of the elevated pH conditions characterizing the low-smectite bentonite clay experiments, Cd^{2+} sorption occurred to a greater extent in these experiments in comparison to high- and medium-smectite bentonite clay experiments.

Summary and Further Insights:

Unlike observations made for Cu^{2+} (see Section 4.2.2.1), Cd^{2+} sorption did not proceed to approximate completion in these experiments. The *extent* of Cd^{2+} sorption was strongly dependent upon interactive effects of bentonite clay substrate composition, microbial metabolic influences, and solution pH. Cd^{2+} sorption maxima in sterile treatments showed variation between bentonite clay substrates, proceeding to ~71% bound in the high-smectite bentonite clay experiments, ~76% bound in the medium-smectite bentonite clay experiments, and ~93% bound in the low-smectite bentonite clay experiments (all values reported for As-augmented treatments). Variation observed in sterile treatments reflects the importance of substrate mineralogy in dictating solution chemistry, including pH, and hence, Cd^{2+} sorptive behaviour. Intuitively, corresponding to the abundance of highly sorbent smectite clay minerals existing in the high-smectite bentonite clay, this clay was be predicted to immobilize the greatest amount of Cd^{2+} of the three bentonite clays investigated; however, the low-smectite bentonite clay immobilized 22% more Cd^{2+} than the high-smectite bentonite clay, highlighting the importance of substrate carbonate content in controlling cationic metal sorption.

Although sorption trends for Cd^{2+} demonstrated strong correlation with solution pH (i.e. in general, Cd^{2+} sorption was enhanced under elevated pH), the influence of pH varied with the bentonite clay substrate being evaluated. While the typical pH-dependence of cationic metal sorption was observed herein, pH alone does not adequately constrain the processes involved. At roughly uniform pH, the amount of Cd^{2+} sorbed by different bentonite clay substrates varied substantially; for instance, at pH ~6.5, Cd^{2+} sorption proceeded to ~76% (high-smectite, T3 with As) and ~90% (medium-smectite, T2 with As). Strict attribution of Cd^{2+} sorptive behaviour to pH would require experimental results indicating that *x*% of the initial Cd^{2+} loading was bound at a given pH, and with set variation in pH, Cd^{2+} sorptive behaviour should respond uniformly, independent of sorbent composition, microbial augmentation, and As-amendment. However, such an observation was not made in these experiments, and underscores the importance of the numerous linked processes which coherently dictate solution and solid phase chemistry, including metal(loid) sorption, within the experimental microcosms. Therefore, while Cd^{2+} sorption displays strong correlation to solution pH when evaluating uptake onto a *single* sorbent substrate (i.e. high-smectite bentonite clay *or* medium-smectite bentonite clay *or* low-smectite bentonite clay), comparison of uptake at uniform pH across treatments and in the presence of different bentonite clay sorbents reveals that Cd^{2+} uptake is influenced by processes external to pH.

Processes operating within sterile microcosms include mineral and amorphous phase dissolution and precipitation reactions, potential complexation with dissolved organic carbon (DOC) in solution, and sorption/desorption reactions; the balance existing between these processes in turn dominates solution chemistry, including pH. Bacterially-augmented microcosms are sufficiently more complex in comparison, integrating processes such as mineral and amorphous phase dissolution and precipitation, sorption/desorption reactions, metal complexation with DOC, aerobic oxidation of acetate, arsenate reduction coupled to acetate oxidation, and certainly numerous other processes. The pH_{PZC} value of sorbent surfaces would also assume an important role in controlling Cd^{2+} sorption, and this property would change with alteration of the solid phase in experimental treatments. Evident in these experiments is the interconnectedness observed between solid and aqueous phase chemistry and microbial metabolic activity (see Figure 5.1), and the observed implications for Cd^{2+} sorption.

4.2.2.3 As Sorption

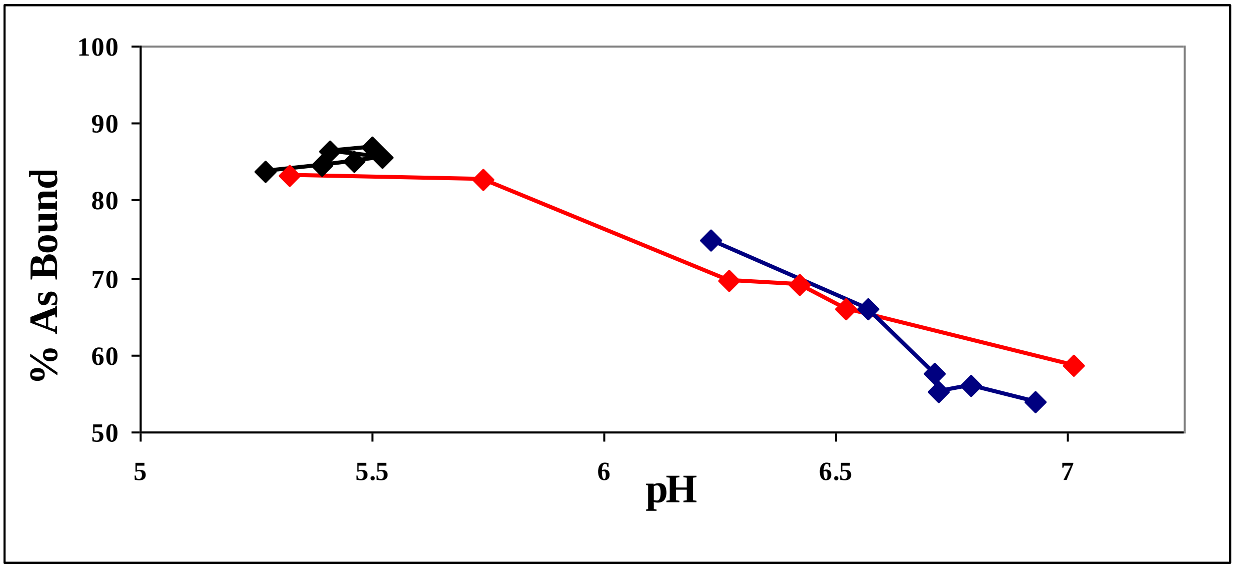


Fig. 4.13 – Bound As versus pH, high-smectite bentonite clay experiments

As sorption to the high-smectite bentonite clay (see Figure 4.13) in T1 showed minimal variation throughout the experimental timeframe. This result is attributed to: a) the minimal change in solution pH observed over time; b) the absence of microbial reduction of *As^{5+} (which would exist in solution as arsenate, AsO_4^{3-}) to the more mobile and labile (Korte & Fernando, 1991, Cummings et al., 1999, Goldberg, 2002, Bissen & Frimmel, 2003, Tufano & Fendorf, 2008, Tufano et al., 2008, Babechuk et al., 2009) *As^{3+} (which would exist in solution as arsenite, AsO_2^-) species; and c) the absence of Fe-reducing bacteria, which can remobilize sorbed As via the dissolution of Fe-oxyhydroxides (Cummings et al., 1999) and other important sorbent phases. In addition, As^{5+} has been shown to form substantially stronger sorption complexes with numerous mineral constituents in comparison to As^{3+} (e.g. Tufano et al., 2008 and references therein), supporting the lack of remobilization observed in the absence of chemical reducing agents or microorganisms capable of reducing As^{5+}. *Note that As^{5+} and As^{3+} will be used in text herein, but refer to arsenate and arsenite, respectively.

In contrast to sterile controls, remobilization of As was observed in bacterially-augmented treatments with time. Remobilization of As in bacterially-augmented treatments displays a strong correlation to solution pH (T2 R^2 = 0.95, T3 R^2 = 0.93), supporting that microbially-induced pH fluctuations can influence As dynamics and partitioning between solid and aqueous phases in the presence of the high-smectite bentonite clay. In addition to the influence of solution pH with regards to controlling As dynamics, the potential for bacterial reduction of As^{5+} also exists (see Equation 4.8). In recent years, several microorganisms, such as *Chrysiogenes arsenatis* (Krafft & Macy, 1998), *Bacillus benzoevorans* HT-1 (Kocar et al., 2006) and *Bacillus selenitireducens* MLS10 (Afkar et al., 2003), as well as a number of known Fe-reducing bacteria, including *Shewanella sp.* ANA-3 (Tufano et al., 2008), *Shewanella putrefaciens* CN-32 (Kocar et al., 2006) and *Geospirillum barnesii* SES-3 (Laverman et al., 1995) have been shown to facilitate the reduction of As^{5+} to As^{3+}. Although 16s rRNA gene sequencing identified no *known* As^{5+} reducers in either enrichment used in these experiments, a substantial proportion of "unclassified" bacterial sequences were obtained for both enrichments (see Section 4.3.1); this fact highlights the potential existence and activity of bacteria capable of As^{5+} reduction, either via respiratory or detoxification mechanisms, in these experiments. Continual advances to current sequence databases as well as further understanding of the diversity of microorganisms capable of arsenate reduction will undoubtedly assist in resolving issues such as this in future studies. Further in-depth characterization of the microbial community, specifically targeting the presence and activation of genes corresponding to As^{5+} reduction via detoxification (*ars* genes) or respiration (*arr* genes) (Saltikov et al., 2005) would be instrumental to confirming the presence and activity of As-reducing bacteria, but were beyond the scope of this research. Additional insights could be gained from the use of molecular level spectophotometric (e.g. molybdenum method; Johnson & Pilson, 1972, Cummings et al., 1999) or synchrotron radiation-based techniques (e.g. X-ray Absorption Fine Structure (XAFS); Cummings et al., 1999; X-ray Absorption Near Edge Structure (XANES); Babechuk et al., 2009) to elucidate As speciation in solution and in association with the solid phase (including via sorption complexes), respectively.

Remobilization of As was most notable from the high-smectite bentonite clay. Differences observed in arsenic remobilization between T2 and T3 over the first two sampling periods are primarily attributed to pH (see Section 4.2.1), such that the more rapid increase in solution pH observed in T3 corresponds to more rapid As desorption. Although no direct experiments were conducted in this thesis to chemically encourage the desorption of sorbed metal(loid)s in an effort to derive the mechanism(s) by which metal(loid) sorption occurred (i.e.

inner-sphere vs. outer-sphere complexation), remobilization of As observed in microbial treatments provides some insight in this regard. Assuming the absence of microbial reduction of As^{5+} to As^{3+} and noting the relatively small magnitude of pH fluctuation characterizing the microbial treatments, it is postulated that As sorption onto the high-smectite bentonite clay occurred, at least in part, via non-specific, outer-sphere complexation. Outer-sphere complexes are known to be sensitive to fluctuations in solution pH and ionic strength (Stumm & Morgan, 1996, Brown et al., 1999, Small et al., 2001, Maurice, 2009), and sorption via this mechanism proposes that a portion of bound As can be readily remobilized with fluctuation in these solution parameters. Independent of the mechanism by which sorbed As is remobilized, these data demonstrate that arsenic bound to natural bentonite clays can indeed become remobilized under changing geochemical conditions resultant of microbial metabolic activity.

Noting the remobilization of As observed herein, implications arise concerning the use of natural and modified clay and clay mineral sorbents for As immobilization, as well as the natural attenuation of As by soil mineral constituents (e.g. Goldberg, 2002, Beaulieu & Savage, 2005, Doušová et al., 2009, Su et al., 2011). Results herein confirm the potential for As sorbed to the high-smectite bentonite clay to be remobilized due to the influence of Fe-reducing bacteria and other microorganisms possessing the capability to modify local microenvironmental pH regimes, induce the dissolution of sorbent mineral phases such as clay minerals and Fe(III)-oxyhydroxides, or reduce As^{5+} to the more mobile As^{3+} species. These results suggest that sorbed metal(loid) stability should indeed be viewed as sensitive to microbial attack, and further support that microbial vulnerability must be taken into consideration when utilizing sorption reactions to control metal and metalloid mobility in environmental, mining, and wastewater treatment applications. Further chemical (e.g. desorption experiments) and spectroscopic (e.g. XANES, EXAFS, Raman spectroscopy, Fourier-transform infrared, wide-angle X-ray absorption) investigation of As-complexed bentonite clay sorbents would provide additional insights into the strength of sorption complexes formed between the mineral substrates and As, perhaps providing an indication of the likely extent to which desorption can occur.

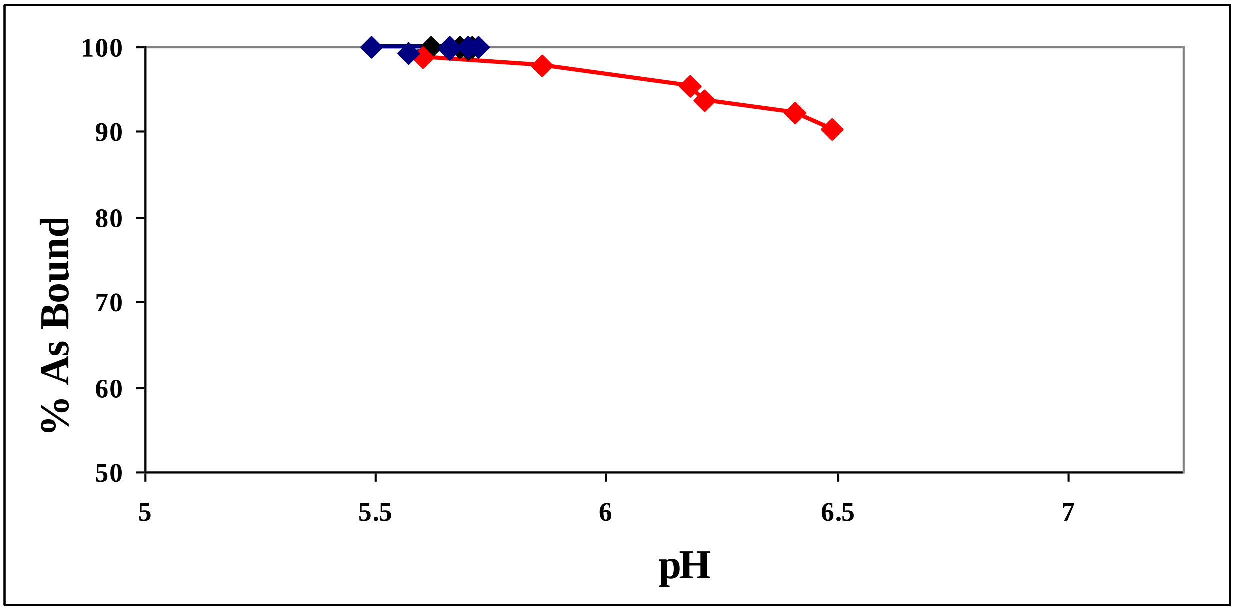


Fig. 4.14 – Bound As versus pH, medium-smectite bentonite clay experiments

As sorption to the medium-smectite bentonite clay (see Figure 4.14) showed minimal variation in T1 and T3, with >99% of the initial As loading remaining sorbed throughout the experimental duration. Solution pH of T1 and T3 remains fairly constant over the experimental timeframe as well, ranging from 5.62-5.71 in T1 and 5.49-5.72 in T3. Noting the apparent trend observed between solution pH and As mobility in the high-smectite bentonite clay experiments (see above), results herein support the contribution of pH in controlling As sorption dynamics; within roughly the same pH range (5.5 +/- 0.25 units), no As desorption was observed in either the medium- or high-smectite bentonite clay experiments. Thus, lower solution pH conditions observed in the T1 and T3 medium-smectite bentonite clay experiments are not conducive to the remobilization of bound As. T3 Fe-reducing bacteria had negligible influence on solution pH in the presence of the medium-smectite bentonite clay and correspondingly showed no influence on As sorption dynamics in these experiments.

Contrarily to T1 and T3, remobilization of bound As is observed in T2. T2 vessels experienced an increase in pH by nearly a full pH unit over the six week experimental timeframe, and As remobilization shows a strong correlation to solution pH (R^2 = 0.93). These results lend credence to: a) the importance of solution pH in controlling As sorption dynamics in the presence of *specific* bentonite clay sorbents; b) the existence of weak, outer-spherically sorbed As complexes; and c) the sensitivity of sorption reactions to solution chemical parameters, and hence, microbial metabolic activity, as discussed above.

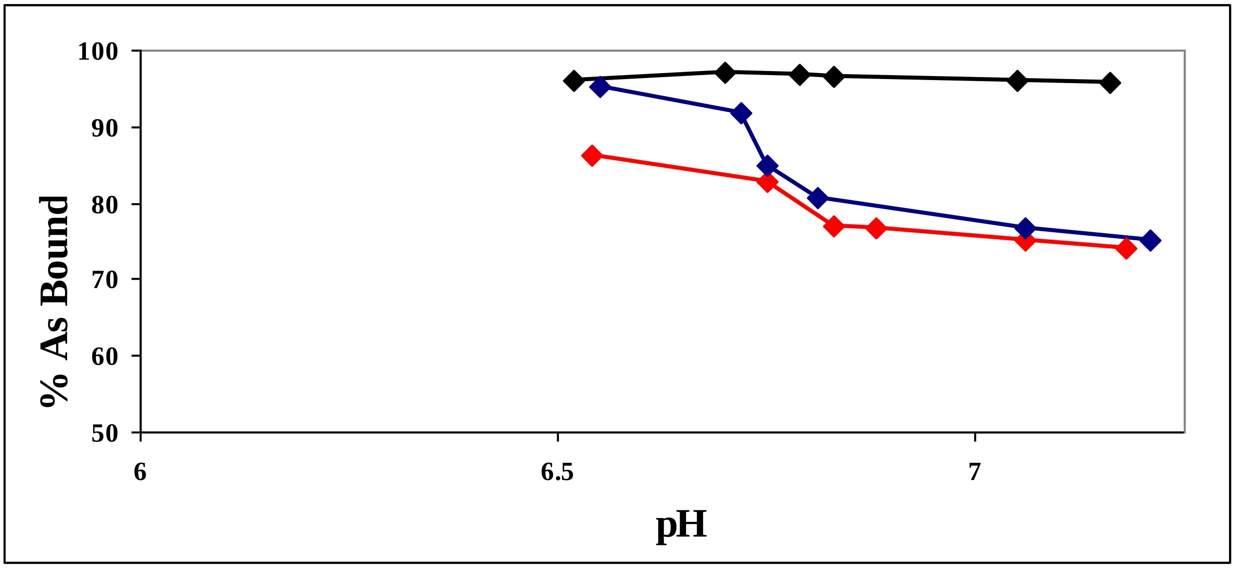


Fig. 4.15 – Bound As versus pH, low-smectite bentonite clay experiments

While solution pH remains fairly consistent between sterile and microbial treatments in the low-smectite bentonite clay experiments (see Figure 4.15), As behaviour differs between bacterially-augmented and sterile treatments. This observation contrasts results from the high- and medium-smectite bentonite clay experiments, which indicated the importance of pH in controlling As sorption/desorption behaviour. The differences in As mobility observed across treatments at roughly uniform pH in low-smectite experiments confirm that As mobility in this case is governed by processes or parameters independent of solution pH. This may be explained by three possible microbially-driven mechanisms: a) bacterial reduction of As^{5+} to the more mobile As^{3+} species; b) microbially-induced remobilization of As associated with the reductive dissolution of amorphous Fe-oxyhydroxides, phases well documented to effectively scavenge As (e.g. Cummings et al., 1999, Kocar et al., 2006, Morin & Calas, 2006 and references therein, O'day, 2006 and references therein, Tufano & Fendorf, 2008, Tufano et al., 2008); and c) enhanced As sorption and retention to amorphous (i.e. Fe-oxyhydroxides and/or organics) material in T1, which is present in greater abundance in the solid phase residues from T1.

Detailed As speciation assays as well as analyses to identify the presence of *arr* and/or *ars* genes corresponding to As reduction would be required to confirm the ability of bacteria from the utilized enrichments to reduce As^{5+} to As^{3+}, and are advised for future experiments of this type. XRD data (see Table 4.3) show relative similarities between T1-T3 with the primary exception identified as the abundance of an amorphous phase, which is greatest in T1. Assuming that a portion of the amorphous material comprising solid phase residues are Fe(III)-oxyhydroxides, which are known for their As-scavenging abilities, the excess abundance of amorphous material comprising solid residues in T1 are postulated to demonstrate a correspondingly higher affinity for As, thereby preventing As remobilization. Importantly, a higher affinity of T1 solid phase for As cannot alone fully explain the release of As observed in bacterially-augmented treatments. Higher sorption affinity for As in sterile treatments would intuitively result in more *extensive* sorption of As in comparison to bacterially-augmented treatments containing less amorphous sorbent material; however, the observed *remobilization* of As in T2 and T3 must result from microbially-induced reductive dissolution of amorphous Fe-oxyhydroxides (see Equation 4.6) and/or the reduction of arsenate to arsenite coupled to acetate oxidation (see Equation 4.8). This observation lends credence to the idea that bacterial metabolic processes can persist and influence system chemistry in experimental microcosms, despite the absence of obvious fluctuations in solution pH (see Section 4.2.1).

Summary and Further Insights:

Although As experimental results displayed correlation between pH and remobilization of sorbed As *from a given bentonite clay sorbent*, the influence of pH varied substantially among bentonite clay substrates. The existence of an additional microbially-coupled mechanism of As remobilization observed in the absence of pH fluctuation was identified to dominate in the low-smectite bentonite clay experiments, and is explained by the reductive dissolution of sorbent amorphous Fe-oxyhydroxide phases and/or the reduction of arsenate to arsenite. In addition, As sorption experimental results demonstrated the importance of solid phase composition on both the extent of As sorption as well as the extent to which As is remobilized in the presence of Fe-reducing bacterial enrichments. In the absence of Fe-reducing bacterial inoculum, negligible remobilization of As was observed from any of the three bentonite clay sorbents evaluated in the principal batch sorption experiments. Bacterially-catalyzed remobilization, however, was observed in all but one augmented treatment (T3 medium-smectite bentonite clay), thereby demonstrating the important role that Fe-reducing bacteria can assume in determining sorbed metalloid stability, and further highlighting the microbially-vulnerable nature of bentonite clay-metalloid sorption reactions.

Sorption of As proceeded to near completion in T1 and T3 of the medium-smectite bentonite clay experiments, while displaying distinct remobilization trends in select other treatments (i.e. T2 and T3, high- and low-smectite bentonite clays, and T2, medium-smectite bentonite clay). Similarly to observations made regarding Cd^{2+} sorption, the *extent* of As sorption was strongly dependent upon the solid phase composition of the bentonite clay substrate (as evidenced by sorption in sterile controls), microbial metabolic processes occurring within experimental treatments, and solution pH; perhaps most importantly, however, are the interactions occurring between these three components that together, define the solid- and solution-phase chemistry of the experimental microcosms.

Sterile treatments bound 86-100% of the initial As loading depending on the bentonite clay sorbent under consideration, which supports the existence of a solid phase compositional control partially influencing As sorption. Despite the strong correlations noted previously to exist between solution pH and As sorption to the high- and medium-smectite bentonite clays, the influence of pH varied substantially when evaluated across bentonite clay substrates and treatments. This variation was manifest in two ways: a) solution pH assumed a non-extant role in controlling As desorption from the low-smectite bentonite clay, as demonstrated by T2 and T3 results; and b) As sorption/desorption to and from the high- and medium-smectite bentonite clays occurred non-uniformly across clay substrates and treatments, despite roughly uniform solution pH observed at specific sampling periods. For instance, at pH ~5.7, T2 high-smectite bentonite clay bound ~83% of the initial As loading, while T1 medium-smectite bentonite clay bound ~100% of the initial As loading.

The collapse of the idea of a primarily pH-dependent mechanism driving As sorption/desorption behaviour when comparing across bentonite clay substrates and microbial treatments is in agreement with conclusions made regarding Cd^{2+} sorption onto bentonite clay substrates; principally, ascribing As sorption/desorption processes strictly to solution pH does not adequately constrain the processes involved. If a pH-dependent mechanism was indeed *solely* responsible for driving As sorption/desorption dynamics in these experiments, across treatments and bentonite clay substrates, one would expect to see: a) pH-dependence dictating As sorption/desorption trends, which was absent in the low-smectite bentonite clay experiments; b) uniform % bound As at set pH; and c) uniform magnitude of As sorption/desorption with set variation in pH. However, results from the experiments conducted herein do not coincide with the aforementioned conditions, thereby underscoring the importance of the multitude of processes affecting solid- and solution-phase chemistry within experimental microcosms, rather than confirming a pH-based control driving As sorption/desorption dynamics.

Within sterile microcosms, solution- and solid-phase chemistry, including parameters such as pH, are controlled primarily by the dissolution and precipitation of mineral and amorphous phases, complexation with DOC, and sorption/desorption reactions. In contrast, a myriad of processes, including the dissolution and precipitation of mineral and amorphous solid phases, complexation with DOC, sorption/desorption reactions, arsenate reduction coupled to acetate oxidation, aerobic oxidation of acetate, and numerous other processes (see Figure 5.1), the delineation of which would require in-depth evaluation of the full metabolome (e.g. Fiehn, 2002, Weckwerth, 2003, Mapelli et al., 2008) characterizing each microcosm, dominate solution- and solid-phase chemistry of bacterially-augmented microcosms. Results of these experiments, especially the non-uniform influence of pH in controlling As sorption/desorption reactions, supports the interconnectedness of microbial metabolic activity with solution- and solid-phase chemistry, and the observed implications for sorbed As stability. Observed As remobilization in the presence of Fe-reducing bacteria proposes the importance of microbial metabolic activity in the context of sorbed metalloid stability, and further highlights the microbially-vulnerable nature of sorbed metalloids.

4.3 Bacterial Community Composition and Viability Confirmation

4.3.1 Bacterial Community Composition

16S rRNA gene sequencing was used to determine bacterial community composition of the base metal mine tailings and Oil Sands composite tailings enrichments prior to the initiation of principal batch metal(loid) sorption experiments. See Figures 4.16 and 4.17, respectively.

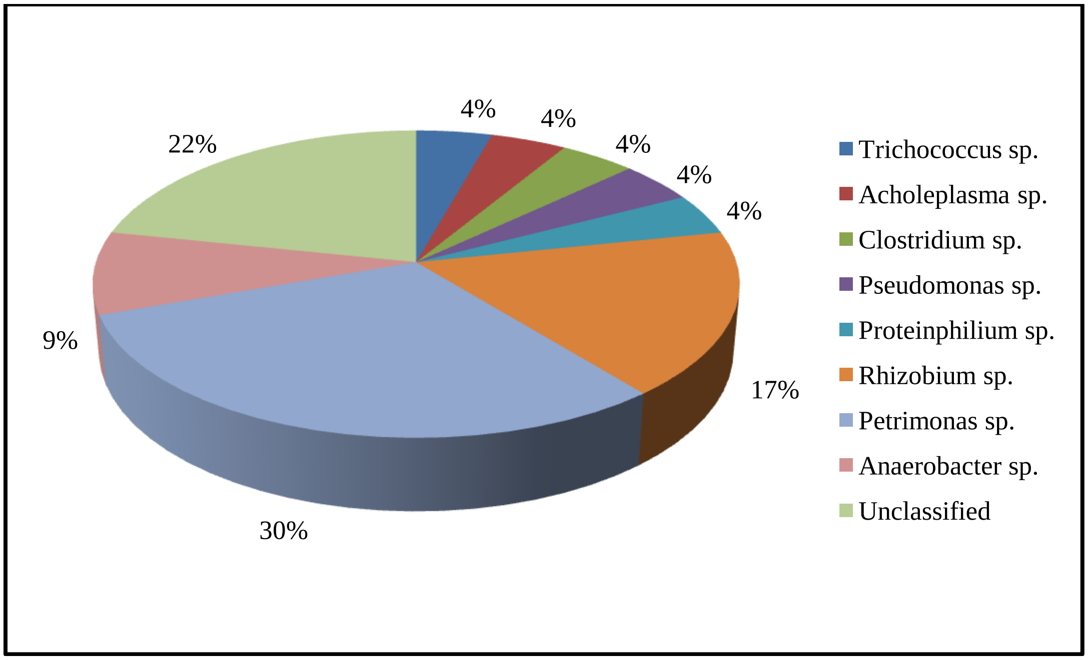


Fig. 4.16 – Bacterial community composition, base metal mine tailings enrichment

16S rRNA gene sequencing identified several potential Fe^{3+} reducers in the base metal mine tailings enrichment. Among these are members of *Clostridium* sp. (Dobbin et al., 1999, Park et al., 2001, Kostka et al., 2002) and *Pseudomonas* sp. (Obuekwe & Westlake, 1982). In addition, members of *Rhizobium* sp. are known to produce siderophores to acquire Fe^{3+} under Fe-deficient conditions (Fabiano et al., 1994, Roy et al., 1994), suggesting the potential contribution of *Rhizobium* sp. to the dissolution of Fe-bearing mineral phases in the principal batch metal(loid) sorption experiments. Unclassified bacterial species represent a large proportion of the sequences obtained for this enrichment, highlighting the potential existence of additional Fe^{3+} reducers or members of other metabolic guilds of importance in the context of the principal batch metal(loid) sorption experiments (e.g. arsenate reducers).

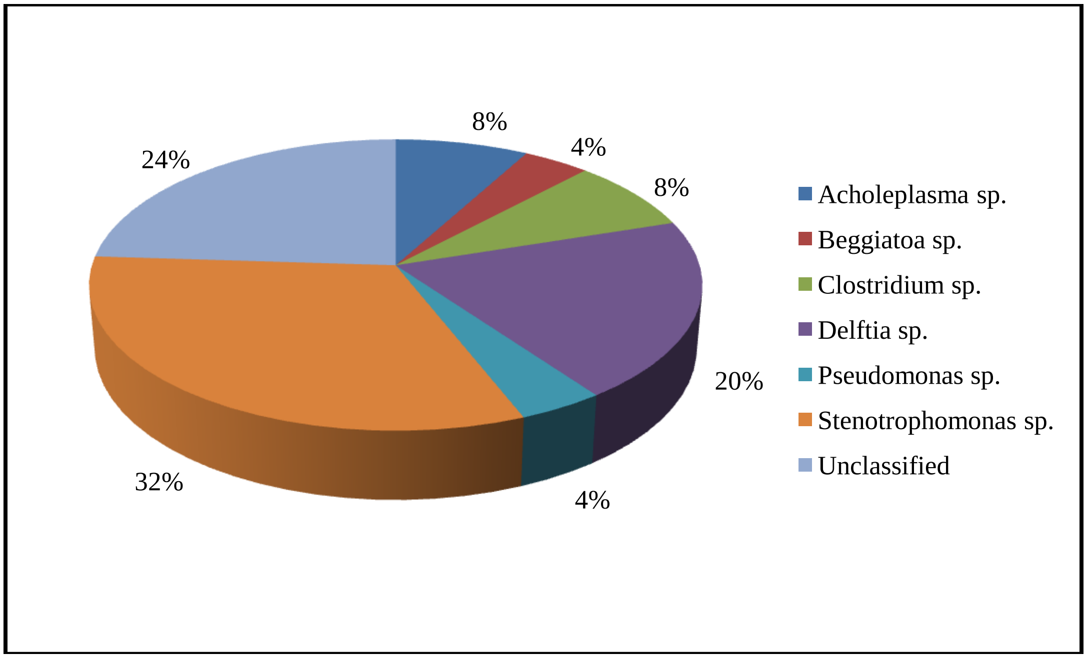
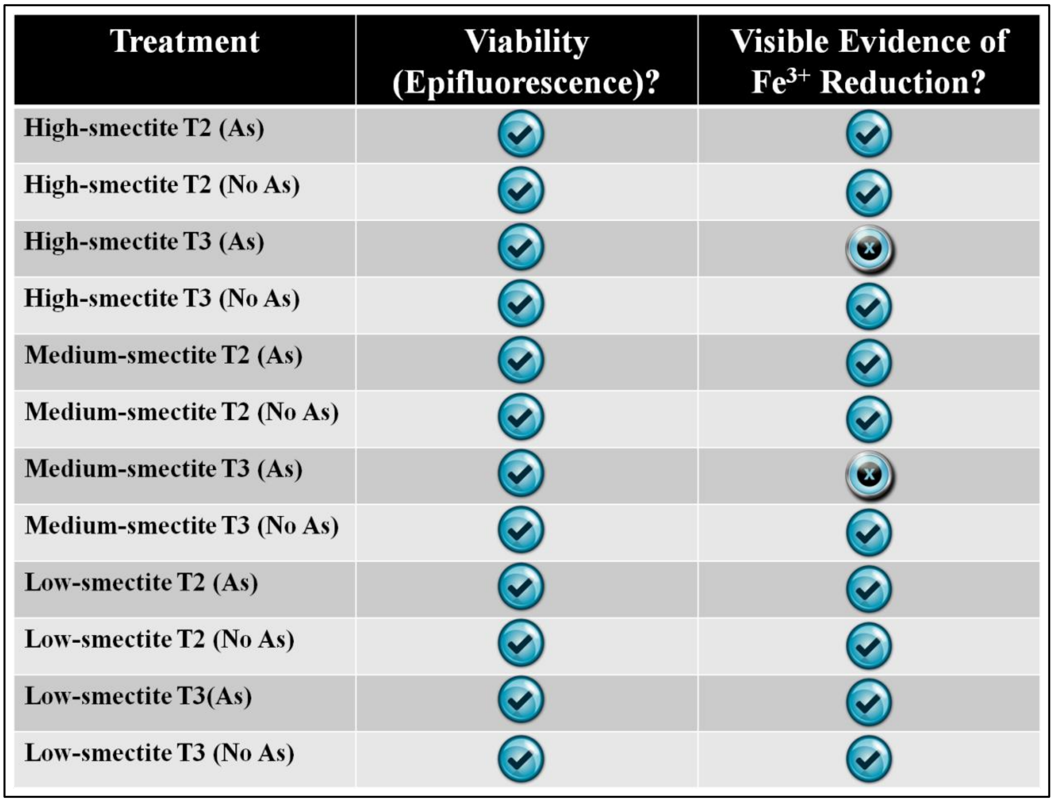


Fig. 4.17 – Bacterial community composition, Oil Sands composite tailings enrichment

Evaluation of the Oil Sands composite tailings bacterial community composition via 16S rRNA gene sequencing identified *Clostridium* sp. (Dobbin et al., 1999, Park et al., 2001, Kostka et al., 2002) and *Pseudomonas* sp. (Obuekwe & Westlake, 1982) as potential contributors to Fe^{3+} reduction in the experimental microcosms. Similarly to sequencing results obtained for the base metal mine tailings enrichment discussed above, a substantial number of "unclassified" sequences were observed in this enrichment; this highlights the potential existence of additional Fe^{3+} reducers or members displaying other metabolisms capable of controlling metal(loid) sorption dynamics within the experimental microcosms (e.g. arsenate reducers).

4.3.2 Post-Experiment Bacterial Viability Results



Treatment	Viability (Epifluorescence)?	Visible Evidence of Fe^{3+} Reduction?
High-smectite T2 (As)	✓	✓
High-smectite T2 (No As)	✓	✓
High-smectite T3 (As)	✓	✗
High-smectite T3 (No As)	✓	✓
Medium-smectite T2 (As)	✓	✓
Medium-smectite T2 (No As)	✓	✓
Medium-smectite T3 (As)	✓	✗
Medium-smectite T3 (No As)	✓	✓
Low-smectite T2 (As)	✓	✓
Low-smectite T2 (No As)	✓	✓
Low-smectite T3(As)	✓	✓
Low-smectite T3 (No As)	✓	✓

Table 4.4 – Post-sorption experiment bacterial viability and Fe-reducing metabolic activity confirmation

Bacterial viability at the conclusion of principal batch sorption experiments was confirmed via LIVE/DEAD® epifluorescence imaging (see Section 3.7; Figure 4.18a-b). Aliquots of experimental slurry sample from each treatment were re-suspended into fresh Fe-reducing media at the conclusion of the sorption experiments, and bacterial suspensions were imaged with the onset of turbidity (see Section 3.4.2). All treatment re-suspensions were confirmed to contain viable bacteria at the conclusion of the experiments. Additionally, confirmation of Fe-reduction in the media was noted by observing colour change and stratification in the growth media in comparison to sterile media volumes (see Figures 4.19a-c), and Fe-reduction was evident in all treatment re-suspensions with the exception of the As-augmented, high-smectite T3 and As-augmented, medium-smectite T3 (see Figure 4.19d). The existence of viable bacteria in all treatments at the end of the six-week sorption experiments is, in itself, an interesting result, confirming the survivability of bacteria from the original enrichments, despite exposure to relatively high concentrations of toxic metal(loid)s. Failure of two of the Oil Sands composite tailings re-suspensions to reduce Fe^{3+} when placed in fresh growth media suggests that iron reducers from this enrichment were more sensitive to the experimental conditions, and were perhaps inhibited due to toxic effects demonstrated by the augmented metal(loid)s. More specifically, toxicological inhibition is predicted to result from As augmentation, as both re-suspensions that failed to reduce Fe^{3+} in fresh growth media were exposed to As in solution during the batch sorption experiments.

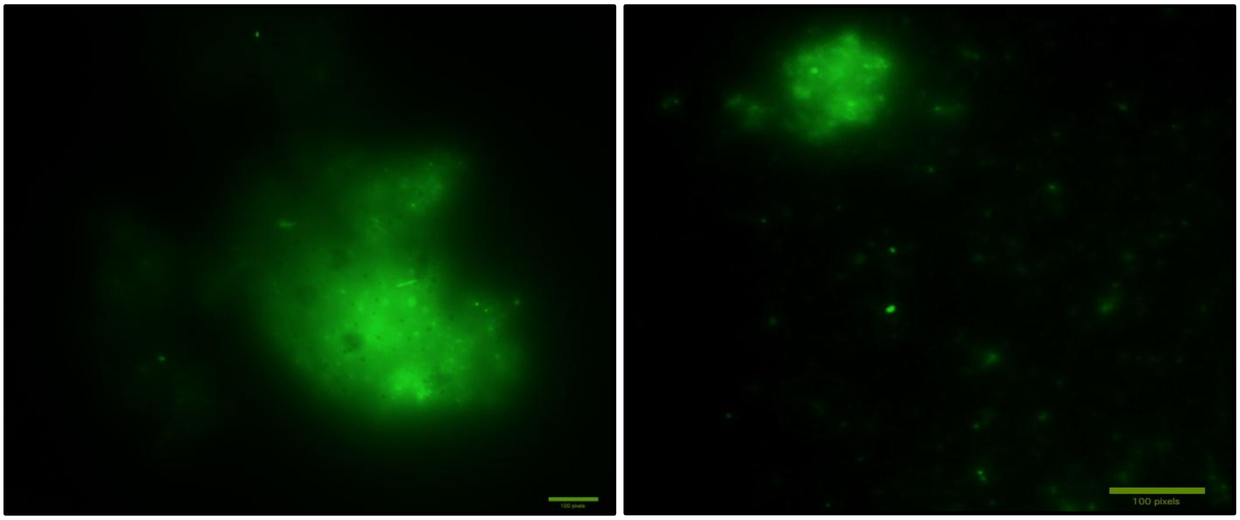


Fig. 4.18 a-b (left to right) – LIVE/DEAD® images showing viable cells embedded in EPS in T2 high-smectite, non-As-amended re-growth and T3 high-smectite, As-amended re-growth.

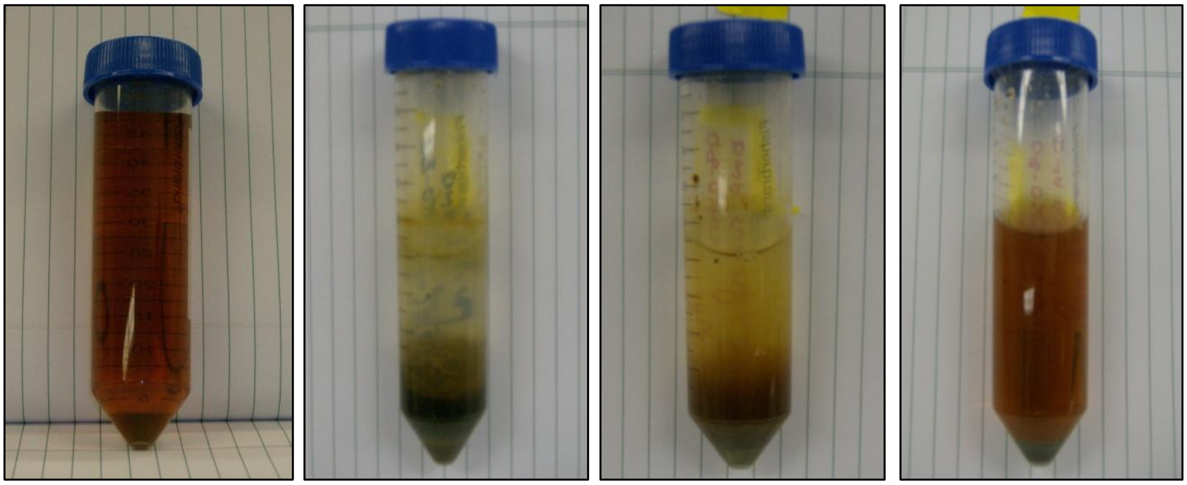


Fig. 4.19 a-d (left to right) – a) sterile Fe-reducing media showing initial orange/brown solution colour due to presence of dissolved Fe^{3+}-citrate; b) Fe-reducing media inoculated with slurry from T2 at the conclusion of principal sorption experiments, showing extreme colour change resultant of Fe^{3+} removal from solution, and stratification due to subsequent re-precipitation of Fe at the base of the centrifuge tube; c) Fe-reducing media inoculated with slurry from T3 at the conclusion of principal sorption experiments, showing moderate colour change resultant of Fe^{3+} removal from solution, and stratification due to subsequent re-precipitation of Fe at the base of the centrifuge tube; d) failed re-growth of T3 slurry suspended in Fe-reducing media, showing no stratification nor change in colour.

5.0 Conclusions

Results of this thesis indicated that environmentally-ubiquitous iron reducing bacteria can indeed influence metal and metalloid sorption to, and the stability of sorbed metal(loid)s (particularly As) on, bentonite clay sorbents. These results underscore the existence of potential limitations to the use of bentonite clay sorbents for the reclamation of mining- and industrially-contaminated wastewater effluents, identifying, for instance, that bentonite clay sorbents should *not* be viewed as a walk-away treatment mechanism for wastewater remediation. Rather, the long-term stability of sorbed metal(loid)s becomes questionable in the presence of Fe-reducing bacteria, supporting that bentonite clay sorbents should be used over short time frames within contained environments and properly disposed of thereafter, or otherwise extracted to retain bound metal(loid)s and prevent future remobilization.

Although augmentation with Fe-reducing bacteria showed no influence on Cu^{2+} sorption in the experiments detailed herein, in general, Cd^{2+} sorption was enhanced, while a hindrance in As sorption and/or encouragement of As desorption with time was observed, thus supporting element-specific sorptive behaviour in these experiments. A suite of metabolically-coupled processes, including the precipitation and reductive dissolution of Fe-oxyhydroxides, dissolution of Fe-bearing clay minerals, aerobic oxidation of acetate, and reduction of arsenate coupled to acetate oxidation, is postulated to drive the changes observed between sterile and bacterially-augmented microcosms with regards to metal(loid) sorptive behaviour. The occurrence of such processes often resulted in definite distinctions existing between the geochemistry of sterile and bacterially-augmented treatments, as reflected in microcosm pH, solid phase composition, and metal(loid) sorptive dynamics, thereby supporting the hypothesized importance of Fe-reducing bacteria in this regard. Table 5.1 provides a succinct description of the observed treatment-specific influence of Fe-reducing bacterial augmentation on metal and metalloid sorption and long-term stability relative to sterile controls.

Table 5.1 – Influence of Fe-reducing bacteria on metal(loid) uptake and stability (relative to sterile controls)

	Cu^{2+}	Cd^{2+}	As
T2 High-smectite bentonite clay	No significant influence on uptake; no observed influence on stability	No influence on initial uptake, **enhanced sorption with time (As^{5+} present);** **Increased initial uptake and enhanced sorption with time (As^{5+} absent)**	**Decreased initial uptake; induced remobilization**
T3 High-smectite bentonite clay	No significant influence on uptake; no observed influence on stability	**Enhanced initial- and time-dependent sorption in presence and absence of As^{5+}**	**Decreased initial uptake; induced remobilization**
T2 Medium-smectite bentonite clay	No significant influence on uptake; no observed influence on stability	**Enhanced sorption over experimental duration**	No change in initial uptake; **induced remobilization**
T3 Medium-smectite bentonite clay	No significant influence on uptake; no observed influence on stability	No significant influence on uptake; no observed influence on stability	No significant influence on uptake; no observed influence on stability
T2 Low-smectite bentonite clay	No significant influence on uptake; no observed influence on stability	Negligible influence on uptake; no observed influence on stability	**Decreased initial uptake; induced remobilization**
T3 Low-smectite bentonite clay	No significant influence on uptake; no observed influence on stability	Negligible influence on uptake; no observed influence on stability	Negligible change in initial uptake; **induced remobilization**

Surprisingly, bentonite clay smectite content showed less importance in explicitly controlling metal(loid) sorption to the substrates than initially postulated. In fact, the high-smectite bentonite clay successfully retained the lowest abundance of As and Cd^{2+} at the conclusion of these experiments, while the medium-smectite bentonite clay retained the greatest abundance of As, and the low-smectite bentonite clay retained the greatest abundance of Cd^{2+} across treatments. Despite the greater relative abundance of smectite comprising the high-smectite bentonite clay, the important role of carbonate minerals in controlling cationic metal sorption was evidenced in the experimental results obtained herein. The low-smectite bentonite clay contained the greatest abundance of carbonate minerals, thereby resulting in elevated solution pH relative to the high- and medium-smectite bentonite clay microcosms, which further acted to enhance Cd^{2+} sorption to this clay. This finding highlights the importance of a thorough understanding of substrate composition when selecting an appropriate sorbent material, and further supports that raw geological materials used for cationic metal immobilization should contain sorbent phases, such as smectites or amorphous Fe-oxyhydroxides, as well as carbonate minerals, which encourage cationic metal sorption by effectively decreasing competition with protons for surface sites. The large carbonate fraction comprising the low-smectite bentonite clay ultimately negated the influence of Fe-reducing bacterial augmentation on cationic metal sorption; contrarily, As desorption was observed, and proceeded independently of solution pH fluctuation, thereby supporting the importance of metabolically-coupled reactions in controlling As sorption dynamics. Comparatively, however, As desorption from the high-smectite bentonite clay proceeded to a greater extent than from the medium- or low-smectite bentonite clays.

Sorption of Cd^{2+} and As to the high-smectite bentonite clay was influenced by augmentation with Fe-reducing bacteria in T2 and T3, while As sorption to the low-smectite bentonite clay was influenced in T2 and T3; Cd^{2+} did not show the same sensitivity to bacterial augmentation in the low-smectite bentonite clay experiments. Cd^{2+} and As sorption in the medium-smectite bentonite clay experiments showed sensitivity to Fe-reducing bacterial augmentation only in T2. These results, as provided in brief in Table 5.1, support that the overall influence of Fe-reducing bacteria on metal(loid) sorptive dynamics followed the sequence high-smectite bentonite clay > low-smectite bentonite clay > medium-smectite bentonite clay, therefore disproving the hypothesized importance of bentonite clay smectite content in controlling the extent of bacterial sensitivity to metal(loid) sorption dynamics.

Remobilization of cationic metals was not observed from any of the bentonite clay sorbents investigated in the principal batch sorption experiments, regardless of Fe-reducing bacterial augmentation and/or As^{5+} amendment. Cu^{2+} sorption proceeded to near completion regardless of bentonite clay substrate and microbial or chemical treatment, inherently supporting that Cu^{2+} sorption occurred independently of these variables. Efficient Cu^{2+} sorption and retention is ascribed to: a) the relative strength of Cu^{2+} sorption complexes derived from the Jahn-Teller distortion; and b) the pH regime characterizing experimental solutions, resultant of the buffering capacity afforded by bentonite clay sorbents, as well as metabolically-coupled acidity consumption. Contrasting Cu^{2+} sorptive behaviour, two differing observations were made with regards to Cd^{2+} sorptive behaviour in bacterially-augmented treatments: 1) bacterial augmentation showed negligible influence on Cd^{2+} sorption dynamics (e.g. T3 medium-smectite bentonite clay, T2 and T3 low-smectite bentonite clay); or 2) Cd^{2+} sorption was enhanced throughout the experimental duration (e.g. T2 and T3 high-smectite bentonite clay, T2 medium-smectite bentonite clay). Cd^{2+} sorptive behaviour demonstrates the importance of numerous intertwining processes, the combined effects of which dictate solution- and solid-phase chemistry within the experimental microcosms (see Figure 5.1).

Bacterial augmentation induced the remobilization of As from all bentonite clays and across treatments investigated herein, with the exception of the T3 medium-smectite bentonite clay experiments, thereby supporting the important role that Fe-reducing bacteria can assume in controlling sorbed metalloid stability. As sorption was correlated to pH when considering sorption to a single bentonite clay sorbent, while non-uniform influences of pH were observed when considering multiple bentonite clay substrates. In addition, a distinct microbially-coupled mechanism of As desorption was observed in the altogether absence of solution pH fluctuation in the low-smectite bentonite clay experiments; this mechanism is postulated to involve the reductive dissolution of sorbent amorphous Fe-oxyhydroxide phases and/or the reduction of arsenate to arsenite. As sorption dynamics support the important interconnectedness of microbial metabolic activity with solution- and solid-phase chemistry (see Figure 5.1), and the observed implications of microcosm chemistry on sorbed As stability. Remobilization of As in the presence of Fe-reducing bacteria underscores the inherent lack of biostability of bentonite clay-sorbed As, and confirms the importance of microbial metabolic processes in dictating the long-term stability of sorbed As. While the two divalent cationic metals investigated herein were not shown to desorb in these experiments, As desorption was observed in the majority of bacterially-augmented treatments.

An important distinction of this study is the incorporation of diverse environmental enrichments of bacteria, rather than the utilization of pure cultures of known iron-reducers (e.g. *Shewanella sp., Geobacter sp.*). While both enrichments influenced sorption dynamics in numerous treatments, referring strictly to the *number* of individual treatments influenced by the presence of each bacterial enrichment, the base metal mine tailings enrichment demonstrated greater influence on metal(loid) sorptive dynamics than did the Oil Sands composite tailings enrichment. Correlations were observed between the presence of the base metal mine tailings Fe-reducing bacterial enrichment and Cd^{2+} sorption to the high- and medium-smectite bentonite clays, regardless of the presence of As^{5+}, and As sorption to all three bentonite clay substrates investigated. In contrast, the Oil Sands composite tailings Fe-reducing bacterial enrichment influenced Cd^{2+} sorption to the high-smectite bentonite clay regardless of As^{5+}-amendment, and As sorption to the high- and low-smectite bentonite clays. Therefore, observing the wider range of influence demonstrated by the base metal mine tailings Fe-reducing bacterial enrichment, the hypothesized differential influence on metal(loid) sorption dynamics characterizing the two distinct bacterial enrichments is supported.

Finally, the hypothesized influence of As^{5+} amendment on cationic metal sorption was only observed in the T2 high-smectite bentonite clay experiments, and was further only evident for Cd^{2+} (see Table 5.1). This effect was manifest as a delay in Cd^{2+} sorption, and was also evident from the pH trend characterizing the high-smectite bentonite clay experiments, where As^{5+} amendment induced retardation in the rate of pH increase. Since similar results were not observed in other treatments, it is largely supported that cationic metal sorption occurred independently of As^{5+} amendment under the experimental conditions detailed herein, and importantly, in the presence of the bacterial communities utilized herein; therefore, this hypothesis is rejected.

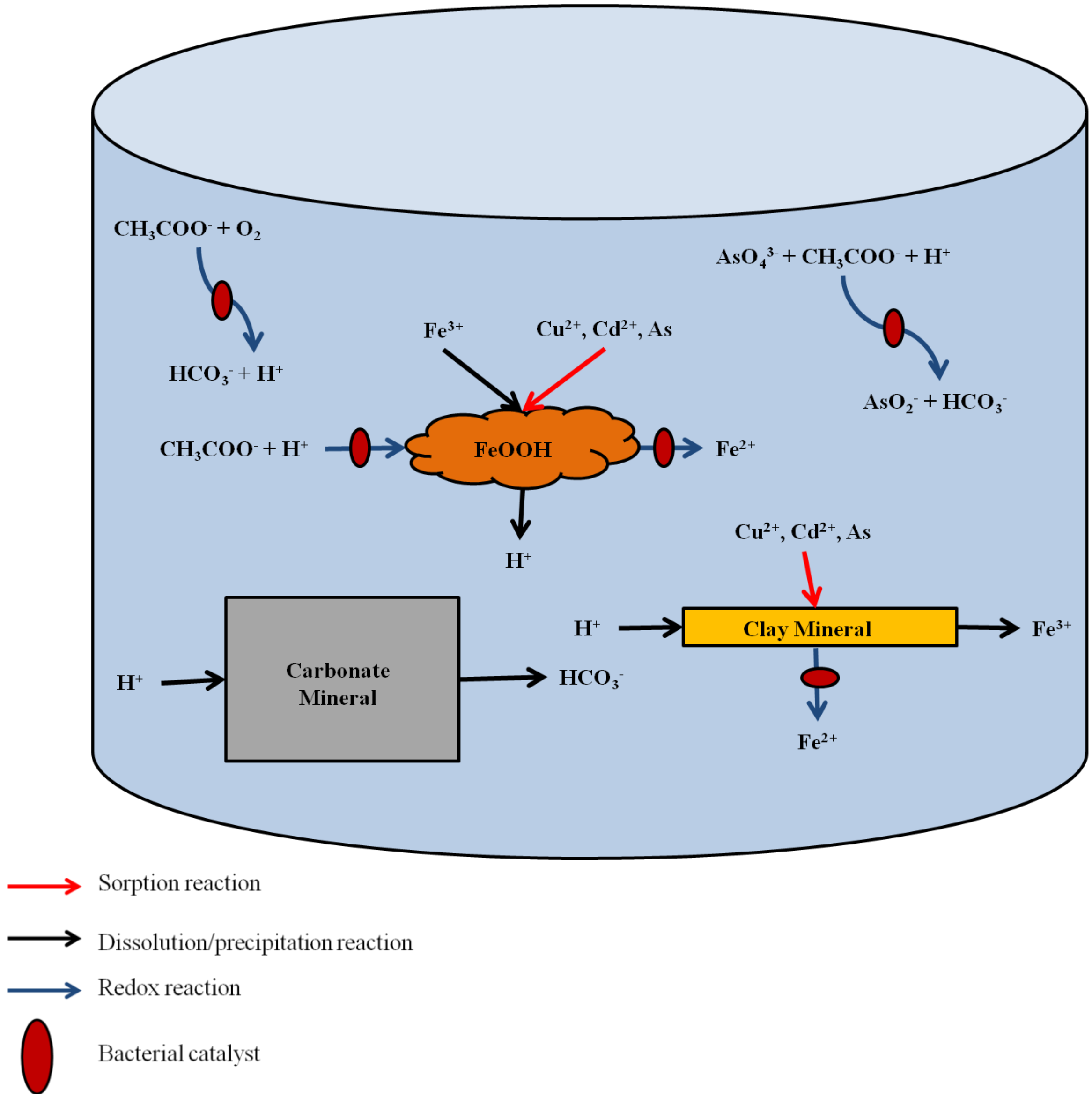


Fig. 5.1 – Dominant processes influencing metal(loid) dynamics, proton balance, Fe cycling, and organic carbon utilization in experimental microcosms.

6.0 Future Research

This thesis investigated the influence of two anthropogenically-impacted enrichments of iron-reducing bacteria on metal and metalloid uptake and stability to bentonite clay sorbents. Although successful in beginning to constrain the importance of Fe-reducing bacteria in this context, this thesis also identifies that room for growth exists. The vast majority of scientific literature related to clay-metal(loid) sorption investigates sorption processes from a strictly abiotic perspective (see Section 1.3.2). Such studies, while instrumental for providing insights into the fundamentals of clay-metal(loid) sorption (related, for instance, to thermodynamics) and determining the utility of clay-based sorbents across varying geochemical conditions, lack real-world applicability, and are thus limited. Primarily, this limitation is derived from the absence of consideration of potential microbial influences on metal(loid) stability and on the longevity of clay-based sorbents in the presence of environmentally-ubiquitous microorganisms, such as Fe-reducing bacteria. Resultantly, the fate of clay-sorbed metals and metalloids when subjected to microbial metabolic activity has remained largely unresolved. This thesis demonstrated the microbially-vulnerable nature of clay-metal(loid) sequestration, particularly concerning As, and supports the undertaking of further research evaluating clay sorbent-bacterial-metal(loid) interactions and dynamics.

Further insights into the sorption-related influence of microorganisms, including Fe-reducing bacteria, may be obtained from further experimentation and research. Noting, for instance, the bacterial influence on system pH dynamics observed in this study, further insights into clay-bacterial-metal(loid) interactions may be uncovered by substitution of the batch-type experimental setup employed in this study with flow-through reactors. While pH represents an important bacterially-mediated variable in these experiments, flow-through reactors would allow additional insights into clay-bacterial-metal(loid) interactions while negating the effects of bacterially-controlled pH fluctuations on metal(loid) dynamics. Further, the use of flow-through reactors would accommodate for negation of pH effects without requiring the use of chemical buffers, which may alter metal and metalloid sorption characteristics. Additionally, flow-through reactors will provide a continual supply of organic carbon, thereby preventing cessation of microbial activity resultant from lack of organic carbon.

This thesis examined a range of environmentally-significant toxic trace elements with varying properties, including two divalent cationic metals of differing hydration character, as well as an oxyanion-forming, metabolically-reducible metalloid. While this thesis has begun to elucidate the influence of Fe-reducing bacterial enrichments on metal(loid) sorption to bentonite clay substrates, further insights will be gained through additional research examining other metals and metalloids of mining and industrial significance, thereby facilitating enhanced understanding of microbial influences on element-specific sorption behaviour and potential competitive sorption interactions.

Contrary to classic batch sorption experimental protocols, which feature typical durations of <24 hours, the experiments carried out in this thesis were run over the course of six weeks to allow for elucidation of the time-dependent influence of Fe-reducing bacterial enrichments on the stability of bentonite clay-sorbed metal(loid)s. Considering the stable, long-term immobilization of toxic trace elements to be the ultimate goal of any treatment mechanism used in metal(loid) sequestration, further insights in this regard may be facilitated via experimentation of even greater duration, particularly when established with the use of flow-through reactors. The influence of Fe-reducing bacteria was observed in this thesis, namely attributed to the dissolution and precipitation of mineral and amorphous phases, and through numerous redox reactions that affect solid- and solution-phase chemistry. Questions then arise as to whether these microbially-catalyzed processes will continue in perpetuity in experiments of greater duration, and importantly, whether these processes will continue to influence metal(loid) dynamics with greater exposure time.

Studies have demonstrated the temperature-dependence of sorption reactions onto clay-based sorbents across the temperature range of 20-90°C (e.g. Kubilay et al., 2007); additional studies examining a range of temperatures typically associated with environmental systems will provide insights into the applicability of clay-based sorbents for use in specific environments and applications. Additional insights may result from evaluation of the influence of enrichments from specific sites at which clay-based sorbents are (to be) employed. Site-specific studies will accommodate for elucidation of the impacts of specific microbial communities on metal and metalloid sorption, and can be adjusted to examine particular environmental conditions pertinent to specific sites or environments. For instance, investigation at low temperatures may show enhanced influence of psychorotolerant bacteria on metal(loid) sorption and stability. Similarly, sorption experiments augmented with microorganisms of competing metabolisms (e.g. Fe-reducers and Fe-oxidizers) may provide more realistic analogue systems to the natural world, allowing for greater depth of understanding of related processes.

Noting the difficulties encountered in this thesis when attempting to image bacteria hosted in a clay mineral matrix via epifluorescence microscopy, advances in epifluorescence imaging techniques will allow for easier confirmation of bacterial viability in experiments targeting clay-bacterial interactions. Advances in nanoscience will also undoubtedly assist in ascertaining and defining the nano-scale processes occurring between iron-reducing bacteria and fine-grained geological materials, including bentonite clay sorbents.

In summary, abundant facets concerning clay-bacterial-metal(loid) interactions in the environment exist which require further in-depth characterization and experimentation to accommodate for an holistic understanding of the potential utility of, as well as limitations to, the use of clays and clay minerals as sorbents for toxic metals and metalloids for the purpose of wastewater remediation.

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