Dissimilatory iron reduction causes lead release from iron-oxyhydroxides.

Arne Sturm
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Dissimilatory Iron Reduction Causes Pb Release from Fe-Oxyhydroxides

By

Arne Sturm

A Thesis
Submitted to the Faculty of Graduate Studies and Research through Earth Sciences in Partial Fulfillment of the Requirements for the Degree of Master of Science at the University of Windsor

Windsor, Ontario, Canada
2006

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ABSTRACT

Iron oxyhydroxides have strong chemical affinities for many trace metals including Pb, in aquatic systems where they often comprise several weight percent of the sediments. During diagenesis, Fe-oxyhydroxides may be reductively dissolved during anaerobic respiration. In freshwater sediments, where sulphate concentrations are low, most Fe reduction is microbially catalyzed. In this enzymatic process termed Dissimilatory Iron Reduction (DIR), Fe (III) minerals are used as terminal electron acceptors coupled to organic matter oxidation. The fate of associated trace metals depends on a number of factors including; secondary mineralization, sorption, and complexation.

In this study we examine the fate of Pb associated with Fe-oxyhydroxides during DIR in the presence of Shewanella putrefaciens 200R and lactate as the sole electron donor. The extent of Fe reduction, determined from the amount Fe(II) extractible from the mineral slurry by 0.5 mol L\(^{-1}\) HCl, was equivalent for both Pb bearing and pure Fe-oxyhydroxides. In contrast, aqueous Fe(II) concentrations in Pb-bearing Fe-oxyhydroxides reactors were significantly higher than those in reactors without Pb. This suggests that secondary mineralization is different in Pb bearing systems and results in less incorporation of Fe(II) into the solid phase. This was confirmed by XRD patterns; the secondary mineral in pure Fe-oxyhydroxides reactors was dominantly magnetite (Fe\(_3\)O\(_4\)) whereas Pb-bearing reactors contained dominantly goethite (FeOOH). The relatively high concentrations of aqueous Pb, despite the abundance of Fe-oxyhydroxides, suggest that aqueous Pb is stabilized in solution by complexation.
CO-AUTHORSHIP

The following thesis contains material from a manuscript that will be submitted to a journal in the near future. The manuscript titled "Dissimilatory Iron Reduction Causes Pb Release from Fe-Oxyhydroxides" is co-authored by A. Sturm, S. Crowe, A. Mucci, B. Sundby, and D.A. Fowle. Laboratory work presented within was performed by the author, with help and guidance from S. Crowe.
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Finally I would like to give thanks to Maxine, Donald and Karla Leslie who gave me amazing financial and moral support, spending many long nights sharing their office and home during the final month of this work.
STATEMENT OF ORIGINALITY

I certify that the thesis herein is a product of my own work, except as denoted in co-authorship. All other work from other people is acknowledged.
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CHAPTER 1 INTRODUCTION

1.1 Overview

Microorganisms are a group of organisms not visible to the naked eye with an enormous diversity. Microorganisms are ubiquitous, and occupy nearly every energetic and geographical niche on this planet (NEWMAN and BANFIELD, 2002). Beyond the remarkable dispersion, they are present in some of the most extreme environments across the planet from high pressure, high temperature hydrothermal vents (LONGNECKER and REYSENBACK, 2001; LUTHER et al., 2001; REYSENBACK et al., 2000) to the extreme cold in Arctic ice (DEMING, 2002; HUSTON et al., 2000). Microorganisms have also been found to tolerate a wide range of pH conditions and can be found in acid mine drainage systems (BOND et al., 2000), acidic hot springs (SIERING et al., 2006) and alkaline spring waters (PEDERSEN et al., 2004).

As a result of their ubiquity and high cell density (NEWMAN and BANFIELD, 2002), microorganisms have a profound impact on the environments within which they live. These impacts can be both passive (e.g. metal diffusion to and sorption on cell walls) and active (e.g. production of O$_2$ by cyanobacteria during photosynthesis). Although their impacts are numerous, the focus here will be upon the role of bacteria in the cycling of carbon via degradation (oxidation) of organic matter in aquatic sediments and aquifers. Microorganisms gain energy (respire) from this redox reaction by catalyzing the oxidation of organic matter (reductant) with a suitable electron acceptor (oxidant). These electron acceptors are used in a sequential order determined by their availability and thermodynamic free energy.
yield (e.g. (Froelich et al., 1979)). In aquatic sediments, the most abundant electron acceptors (listed in order of decreasing free energy yield) are: oxygen (O$_2$), nitrate (NO$_3^-$), manganese oxyhydroxides (e.g. MnO$_2$), ferric iron oxyhydroxides (e.g. the mineral goethite, Fe(OOH)), sulfate (SO$_4^{2-}$), and carbon dioxide (CO$_2$). In turn, zones of electron acceptor predominance can often be observed in sediments and aquifers. Due to both their ubiquity (as coatings and physically discrete phases (Davisson, 1992; Murray, 1979)) and abundance in near surface geologic environments, Fe- oxyhydroxides often play an important role in oxidizing organic matter (Aller et al., 1986; Canfield et al., 1993; Roden and Wetzel, 1996). Organic matter usually takes the role of the electron donor and is supplied in many different forms in natural environments. A facultative anaerobe like Shewanella is able to oxidize many different forms of organic matter (formate, lactate, amino acids, H$_2$ and Pyruvate) (Nealson et al., 2002) using various electron acceptors as indicated above.

Fe is one of the most common elements in the earth's crust. With an abundance of 6 - 7 %, (Faure, 1998) 66

(Figure 1), Fe is the 4th most common element (Press and Siever, 1986). Under modern near surface conditions, Fe occurs predominantly in oxidized valence states including divalent ferrous Fe (Fe(II)) and trivalent ferric Fe (Fe(III)) (Schwertmann and Cornell, 1996). By mass in the crust, most Fe occurs in the form of solid oxides, sulphides and silicates. Some of the most common include: magnetite (Fe$_3$O$_4$), goethite (FeO(OH)), ferrihydrite (Fe(OH)$_3$), hematite (Fe$_2$O$_3$), pyrrhotite (Fe$_{1-x}$S) and pyrite (FeS$_2$) (Schartmann and Cornell, 1996). When
found in near-surface environments, these minerals often have high surface-area to volume ratios, which makes them very reactive to constituents in the surrounding aqueous medium (SCHWERTMANN and CORNELL, 1996; STUMM and MORGAN, 1996).

Fe minerals are effective solid-state redox buffers (HERON and CHRISTENSEN, 1995). Their high abundance, uniform distribution in sediments and aquifers and their reactive surfaces, allow Fe minerals to react rapidly with the aqueous phase. The ability to accept or donate electrons makes them an excellent candidate to buffer prevailing redox conditions. Indeed, the redox potential of many aquatic environments is poised by the Fe(II)/Fe(III) redox couple (e.g. CROWE et al., Submitted)). As noted above, reduction of Fe-oxyhydroxides becomes favourable in aquatic sediments when more energetically favourable electron acceptors become depleted. Iron reducing conditions commonly develop in most freshwater and marine sediments and aquifers receiving moderate supplies of organic matter.

Both the tendency for Fe to buffer the redox state of sediments and aquifers, as well as the strong affinity of Fe-oxyhydroxides for P and many trace metals, suggest a close link between Fe redox cycling and P and trace metal mobility and distribution in the environment. For example, it has been shown that P is released from sediments under Fe reducing conditions (BOSTROM et al., 1988). On a larger scale, it has been shown that a P anomaly above the redoxcline in the Black Sea developed due to redox cycling of Fe (SHAFFER, 1986). It has been shown that trace metal (As, V) release from resuspended sediments is minimized
by the oxidation of Fe(II) and Mn(II) and the reprecipitation of oxyhydroxide minerals (SAULNIER and MUCCI, 2000). Recent studies have shown that a suite of trace metals are released from sinking particulate matter encountering Fe and Mn reducing conditions in estuarine waters (HAMILTON-TAYLOR et al., 2005). Another recent study has shown a direct link between seasonal cycling of Fe-oxides associated with roots in a wetland and Pb mobility (SUNDBY et al., 2005). In their study, Sundby et al. noted that the presence of organic ligands would be required to stabilize aqueous Pb (at the concentrations they observed) in the presence of abundant Fe-oxyhydroxides. Although these field-based studies provide a very good qualitative description of the relationship between trace metal and P cycling and Fe redox transitions, detailed laboratory studies can provide more fundamental mechanistic information. Such studies have been conducted to further elucidate the relationship between P (RODEN and EDMONDS, 1997), Ni, Co, Cr, Al (BOUSSERRHONE et al., 1999; FREDRICKSON et al., 2001; ZACHARA et al., 2001), Zn (COOPER et al., 2005; COOPER et al., 2000; COOPER et al., 2006) and bacterial Fe reduction. The objective of this study was to examine the interplay between Pb and bacterial Fe reduction using laboratory experiments to further the mechanistic understanding of the system.

1.2 Background

1.2.1 Pb in the Environment

Lead (Pb) is a heavy metal that at low concentrations can be toxic to biological organisms in aquatic systems. The toxicity of Pb is related to the
impairment of calcium-dependent intracellular processes (SCHANNE et al., 1989), and, in addition, stimulates the formation of inclusion bodies that may translocate the metal into cell nuclei and alter gene expression (KLEIN and WIREN, 1993). Accumulation of Pb in the sediments of lakes, rivers, flood plains, estuaries and oceans can occur via biological (e.g. biosolid degradation) and geological processes (e.g. mineralization, sorption, particle deposition) (GALLON et al., 2004; GALLON et al., 2005). The ultimate fate of these metals is difficult to constrain and predict, as small changes in pH, redox, or physical conditions can re-mobilize large fractions of these metals from sediments into the overlying waters (AMOUROUX et al., 2003; SUNDBY et al., 2005).

Perhaps more important is that heavy metals such as Pb can not be broken down or remediated in situ, as is possible for many organic contaminants; metals are persistent. Therefore, it is crucial to improve the conceptual and mathematical models of heavy metal cycling, so that metal fluxes in the environment can be better predicted and controlled. By improving predictions of residence times, these models will allow a better understanding of the buffering capacity of an environment.

1.2.2 Association of Pb with Fe minerals

Adsorption and desorption of organic and inorganic species to particles plays an important role in the environmental distribution of these species. For example, the predominant pathway for the removal of trace non-volatile constituents from water is by adsorption and coprecipitaion with particles (BUFFLE
AND DEVITRE, 1994). A critical factor in adsorption and desorption processes is surface area, as the number of sorption sites is directly proportional to the surface area. Small particles have a larger surface-area to volume ratio, which means for a given volume and consequently mass, there is more surface area for smaller particles of the same substance. This relationship is described for spherical particles by the following relationship:

\[
\frac{A}{V} = \frac{3}{r}
\]

where \( A \) is Area, \( V \) is Volume and \( r \) is the radii.

Surface reactivity arises from the presence of ionizable, amphoteric functional groups at the surface of a particle, which can protonate and deprotonate, and bind cations and anions. The surface charge of oxide and oxyhydroxide minerals develops primarily from the ionization of these functional groups and other surface chemical reactions. On Fe-oxyhydroxide surfaces, the charge is therefore pH dependent, with the surface carrying a net positive charge at low pH conditions and a net negative charge at higher pH conditions. The presence of this surface charge results in the development of an electrical double-layer in adjacent solutions. Several conceptual models have been developed to describe the nature of this double-layer, including the constant capacitance model (CCM), the diffusive double layer model (DLM), and the triple layer model (TLM) (BUFFLE and HORVAI, 2001). The important concept drawn from these models is that metal cations can sorb to a Fe-oxyhydroxide surface in two ways: by forming a chemical covalent bond with a functional group that acts as a Lewis base, or by accumulating near the surface due to electrostatic forces that arise from the net surface charge of the
particle. While chemical sorption is in many cases very much dependent upon pH changes, sorption of some metals, like the alkali and alkali earths onto Fe-oxyhydroxides, is also greatly influenced by an electrostatic component of the sorption mechanism. Such electrostatic adsorption only occurs when the sorbate has the opposite charge of the sorbant surface. For cations, this means that electrostatic sorption only occurs at pHs more positive than the zero point of charge (zpc) where the oxyhydroxide surface has a net negative charge. Metals like Pb and Cu adsorb quite effectively (~50%) at pH levels that produce a highly net positive charged surface and are more than 95% adsorbed at the zpc.

Quantifying cation sorption in natural systems is never a simple process. There are many reactions (e.g. coating by organic matter) that alter the nature of particle surfaces and it is not always obvious which ions, positive or negative, will adsorb to a given surface. In many cases, negatively charged ligands complex metal ions and occupy positively charged surface sites on particles. In this event, metal ions adsorb indirectly to a positively charged surface through ternary reactions. In some instances, the cations may also get incorporated or substituted into the structure of the Fe-oxyhydroxides; this happens by the concomitant formation of surface precipitates during the sorption of cations and is therefore known as coprecipitation. By further altering the internal structure and therefore the surface properties, this process makes it even harder to determine the fate of the cation. Therefore, it is necessary to use detailed surface complexation models (SCMs) and state-of-the-art spectroscopic techniques to develop a mechanistic understanding of sorption processes. A review of such techniques is beyond the
scope of this chapter and the reader is referred to the following references for more details (Benedetti et al., 2002; Brown et al., 1999; Ponthieu et al., 2002).

1.2.3 Fe Cycling

As discussed above, Fe is important in naturally occurring redox reactions (Buffle and Devitre, 1994; Hering and Stumm, 1990; Stumm and Sulzberger, 1992). For example, in the water-column of lakes, Fe is mainly present as either Fe(II)aq or suspended Fe(III)-oxyhydroxides, depending upon redox potential. This is due mainly to the dramatically different solubility of the Fe²⁺ and Fe³⁺ ions.

The insoluble nature of Fe³⁺ causes precipitation of Fe(III) oxyhydroxides that sink towards the sediment-water interface by gravitational forces (Figure 2). As these Fe minerals move through the water column, they adsorb metals, nutrients, and organics (Stipp et al., 2002; Tessier et al., 1996) which can subsequently be sequestered during sedimentation, thereby preserving geochemical signatures of the water. In the sediment, Fe(III) in these oxyhydroxides may be used by bacteria as a terminal electron acceptor. The bacteria use the Fe(III) in a respiratory process coupled with the degradation of natural organic substances that are present in the sediments and water column. The production of energy in such a fashion is termed dissimilatory Fe reduction (DIR) (Lovley, 1991). In this process, organic carbon is oxidized to CO₂ which then dissolves into the aqueous phase. This dissolved CO₂ may escape into the water column and causes an increase in sediment porewater HCO₃⁻ and therefore alkalinity. This increase in porewater HCO₃⁻ may then react with porewater metals to form authigenic secondary
minerals, such as rhodochrosite (MnCO\(_3\)) or siderite (FeCO\(_3\)) (Kostka et al., 1992; Zachara et al., 1998; Zachara et al., 2001; Zachara et al., 2002). Thus, Fe mineralogy can preserve a record of sedimentary redox conditions.

During the reduction process, via electron transfer to Fe(III), the Fe-oxide tends to dissolve or restructure to accommodate the accumulating Fe(II) (Hansel et al., 2003; Lovley, 1991; Zachara et al., 2001). During this process, the Fe(II) can be released into the sediment porewaters and subsequently the overlying water column, along with all the metals and nutrients which were adsorbed (Tessier et al., 1996) to the Fe(III) mineral prior to dissolution. In the sediment, the reactive Fe(II) will interact with other dissolved constituents such as CO\(_2\), sulphur, oxygen, and phosphates, forming a variety of secondary minerals (Zachara et al., 2001). As Fe(II) diffuses upwards towards the water column, it may be reoxidized by O\(_2\) or biologically by microbes coupled with alternative oxidants depending on the penetration depth of molecular oxygen. The oxidation of Fe(II) causes the precipitation of amorphous Fe(III) oxyhydroxides resulting in a complete redox cycle.

1.3 Conclusions

Although there is ample evidence linking trace element and iron cycling in the environment, the debate remains over the precise nature of the mechanisms involved. In order to develop a more quantitative understanding of how Fe-mineral phase transformations impact the distribution of trace metals, experimental studies suitable to delineate specific mechanistic pathways are necessary. The intent of
this thesis is to determine the impact of microbial Fe-reduction on the redistribution of the trace metal Pb. To this end, experimental incubations are conducted using environmentally relevant microorganisms and Pb substituted Fe-oxyhydroxides. These experiments were designed to determine:

1. If Pb concentrations used in this study have a toxic effect on the facultative anaerobe Shewanella.
2. How Pb will redistribute between mineral and aqueous phases during microbial Fe-reduction of Fe-oxyhydroxides.
3. If the presence of Pb, either sorbed or coprecipitated to Fe-oxyhydroxide, has an effect on the mineralization products formed during microbial Fe-reduction.
1.4 References


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Figure 1: Composition of the Earth’s Crust

- Silicon, 28.0%
- Oxygen, 46.0%
- Aluminum, 8.0%
- Iron, 6.0%
- Magnesium, 4.0%
- Calcium, 2.4%
- Potassium, 2.3%
- Sodium, 2.1%
- Other, 1.8%
Figure 2: The Fe Cycle (J. Kostka & K. H. Nealson)
CHAPTER 2 THE FATE OF PB DURING THE RESPIRATION OF FE-OXYHYDROXIDES

2.1 Introduction

Lead contamination impacts a diversity of environments and ecosystems ranging from fresh waters to soils, and from aquatic microorganisms to terrestrial forests (VERMILLION et al., 2005). In these settings the toxic effects of Pb are well documented at all levels of the ecosystem (XINARAS, 1992). Elevated levels of Pb are attributed to a variety of anthropogenic sources, including paint use, gasoline combustion, and industrial production (VERMILLION et al., 2005). Evaluating the environmental impact of such widespread contamination requires a detailed understanding of the cycling of Pb within diverse environments and the factors governing the mobility of Pb and its distribution among various reservoirs.

As Pb is a trace component, even in most contaminated environments, it rarely reaches concentrations high enough in aquatic environments to precipitate mineral phases (BUFFLE and DE VITRE, 1994). Therefore, like all trace non-volatile components, the aqueous concentrations of Pb are limited due to scavenging by particulate matter comprised of more abundant components including; organic debris, microorganisms, and inorganic particles. Sedimentation of this particulate matter constitutes the dominant removal mechanism of trace constituents from aquatic systems. Contrary to this, in acid mine drainage Pb can reach high levels and precipitates out of solution. As such, a thorough understanding of the interactions between Pb and this particulate matter is paramount for predicting the fate and residence times of anthropogenic Pb.
Natural inorganic particulate matter often contains an abundance of Fe-oxyhydroxides. These Fe-oxyhydroxides are effective sorbents of Pb due to both the strong chemical affinity of Pb for oxyhydroxide surfaces and the characteristically large surface area to volume ratio of Fe-oxyhydroxide particles. Particulate Fe-oxyhydroxides are stable under the circumneutral aerobic conditions that are characteristic of many sub-aerial aquatic environments. However, once these Fe-oxyhydroxides are incorporated into soils and sediments they may be intermittently subjected to oxidation-reduction processes that will induce precipitation and dissolution, respectively (HERING and STUMM, 1990).

In many soils and sediments Fe(III), in the form of oxyhydroxides, is the most abundant electron acceptor and therefore, buffers soil and sediment redox conditions (HERON and CHRISTENSEN, 1995). While oxygen is supplied by molecular diffusion, convection and bioturbation to water saturated soils and sediments, the presence of organic matter causes heterotrophic aerobic respiration which tends to consume O₂ more rapidly than it can be supplied (FROELICH et al., 1979). Further respiration proceeds using terminal electron acceptors of decreasing free energy yield (NO₃⁻, Mn(III/IV), Fe(III)) (FROELICH et al., 1979). The reduction of Fe(III) to Fe(II) during respiration causes the biologically induced reductive dissolution of Fe-oxyhydroxides and the formation of secondary mineral species including magnetite, siderite and vivianite, depending on the specific conditions (ZACHARA et al., 2002). The fate of Pb and other trace metals associated with Fe-oxyhydroxides during DIR is therefore a complex function of these biogeochemical reactions.
To a first approximation, one might expect that the reductive dissolution of trace metal bearing Fe-oxyhydroxides would cause the release of Pb and other associated trace metals to surrounding waters. Numerous field-based studies have shown that Pb may become mobile under reducing conditions. For example Taillefert et al (2000) found organic matter, Fe-oxide particulates, and elevated levels of Pb associated at the oxic-anoxic boundary of a eutrophic lake. They concluded that the elevated Pb concentrations at this boundary were due to the scavenging ability of the precipitating Fe-oxide minerals and their dissolution under suboxic conditions. Another study demonstrated that fluxes of $^{210}$Pb from sediments to overlying waters develop when the sediment water interface becomes suboxic (Benoit and Hemond, 1990). Sundby et al, 2005, were the first to conduct direct in situ measurements of Pb(II) in the pore water of salt marsh sediments, and found that the annual cyclic mobilization of Pb is due to the oxidation-reduction process controlled by the decay and growth of roots.

Detailed laboratory studies have also been conducted to determine the fate of numerous trace metals associated with Fe-oxyhydroxides. Zachara et al, 1998, determined that there was no difference in the reducibility of synthetic and geological Fe$^{3+}$ minerals, and that the primary factor in determining the secondary mineral suite is the rate and magnitude of Fe(II) supply reacting with residual oxide and other ions (Zachara et al., 2002). These findings were then applied to the study of Co and Ni release from Fe(III)-oxide and it was found that DIR of Fe(III)-oxides did increase the aqueous concentrations of both elements, even over the release of Fe(III) (Zachara et al., 2001).
Extensive work has been done on Zn, with respect to sorption behavior and redistribution abiotically, as well during dissimilatory Fe-reduction. The importance of Zn immobilization into 0.5M HCL-insoluble nano-magnetite by Fe-reducing bacteria was illustrated by Cooper et al., 2000. The above study indicated that the nature of the oxide surface was an important control in the formation of secondary minerals and such formation has a profound impact upon trace metal distribution. The results of a study in 2005 (Cooper et al., 2005) suggested that mineral composition (not just the amount of reducible Fe but other constituents as well) is important for the coordination of the Zn when it binds to the secondary minerals. From the above result, the conclusion that laboratory studies with synthetic minerals do not represent natural systems was drawn. Results from Fredrickson et al, 2001, demonstrated that Ni^{2+} in aqueous solution can inhibit DIR, but in the presence of ligands, like humic acids, may be immobilized and coprecipitated into the crystal structure of biogenic magnetite. These effects are demonstrated again in more detail and for a suite of other trace metals by Cooper et al., 2006.

Previous work on the coprecipitation of base metals with ferrihydrite yielded a selective solubility of metals (Pb>Cu>Zn>Co>Cd) (Martinez and McBride, 1998a; Martinez and McBride, 1998b; Martinez and McBride, 2001). During coprecipitation the addition of organic ligands and a higher concentration of Fe^{2+}_{aq} resulted in a greater scavenging ability of heavy metals (Martinez and McBride, 2001; Violante et al., 2003). Ford et al, 1997, studied the transformation of ferrihydrite to goethite and hematite, and postulated that Mn and Ni were incorporated into the crystalline structure, while Pb and Cd were not.
Most studies examining the fate of trace metals during iron reduction do not adequately examine the distribution of the trace metals in the pore and bulk water or secondary mineralization products (Sundby et al., 2005). Furthermore, most of these studies fail to consider the potential role of biologically produced organic ligands (Violante et al., 2003). This study will examine the fate of Pb, associated with Fe-oxyhydroxides during biologically induced Fe-oxyhydroxide reductive dissolution. A novel suite of techniques, including a recently developed scheme of selective extractions to determine the partitioning of Pb between various potential reservoirs was applied.

2.2 Experimental

To address the objective of this study, the fate of Pb during DIR, both Pb bearing and Pb free Fe-oxyhydroxides with similar physical properties were synthesized. These oxyhydroxides were incubated with environmentally relevant Fe-reducing microorganisms, specifically Shewanella putrefaciens 200R. A variety of techniques to determine the partitioning of Pb between the solid and aqueous phases as a function of time were then performed.

2.2.1 Microbial Culture

The strain used in this study, Shewanella putrefaciens 200R, was obtained from J. Haas (University of Western Michigan). S. putrefaciens 200R was originally isolated from a crude oil pipeline in Alberta, Canada (Obuekwe and Westlake, 1982). Strain 200R is a rifamycin-resistant anaerobe that can respire using several
redox couples, including Fe(III)/Fe(II) (Dichristina and DeLong, 1994). The culture was maintained as a glycerol stock at -80°C. Experimental cultures were grown by plating the glycerol stock on Trypticase Soy Agar (Difco, Detroit), inoculating single colonies into 10 ml tubes of Trypticase Soy Broth (TSB) amended with yeast extract and incubating aerobically at 32°C for 18 hrs. The 10 ml seed cultures were used to inoculate 0.5 L of TSB, also amended with 5 g of yeast extract that was then incubated at 32°C until the culture reached the late log phase (18 hrs). Cells were harvested by centrifugation at 3000 g and washed 5 times in aerobic experimental media (Table 1), and once in anaerobic media, both of which contained no electron donors. The initial cell density was 10^8 cells ml^-1 determined by DAPI staining and direct counting. Sodium lactate was provided as the sole electron donor; it was filter sterilized (0.2um) and added to the experimental media in a sterile laminar flow hood to a concentration of 24 mM.

2.2.2 Preparation of Iron Substrates

Pb bearing and Pb free Fe-oxyhydroxides were synthesized by controlled hydrolysis of 0.04 M ferric chloride solutions (Martinez and McBride, 2001). Pb acetate (2.78x10^-5 M) was added to 0.2 M ferric chloride solutions. These solutions were titrated at a rate of 0.8 and 0.2 ml min^-1 with 1M KOH (Man-Tech Associates Inc. PC-Titrator) to a pH of 7.00. The solution pH was initially at pH 2.31 due to acidity generated by dissolution of the ferric chloride salts. Pb free Fe-oxyhydroxides were synthesized in the same way without the addition of Pb acetate to the starting solutions. The Fe-oxyhydroxide was washed by
centrifugation and re-suspended 5 times in 18 MΩ reverse-osmosis-deionized-water (Milli-Q). The chloride concentration of the final wash solution was less than 0.1 ppm in the final wash as determined by ion chromatography (Dionex). The Fe-oxyhydroxide was then tested for Pb content by ICP-MS (Thermo X7 series, Serial No. 2580 equipped with a standard conical nebulizer calibrated against Inorganic Venture Standards, which are traceable to National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA)), which was less than 0.5 ppb, to determine and evaluate the reagent blanks and methods used to prepare the sample. The Fe-oxyhydroxides were then washed 2 times with minimal media that contained 24 mM lactate. Although Martinez and McBride (2001) obtained pure ferrihydrite during their synthesis, XRD spectra of the Fe-oxyhydroxide synthesized in this work revealed the presence of akaganeite (β-FeOOH), a crystalline Fe-oxyhydroxide that can form in the presence of high concentrations of Cl⁻ (Figure 3) (Reguer et al., 2006; Schwertmann and Cornell, 1996; Zachara et al., 2002).

2.2.3 Mineral Incubations

Acid-washed 50 ml polycarbonate Falcon tubes were used as reaction vessels and each tube contained 50 mmol l⁻¹ Fe, in the form of Fe-oxyhydroxide. Twenty-three of the tubes contained Pb bearing oxyhydroxides and were inoculated with approximately 40 mL of microbe bearing (10⁸ cells ml⁻¹) minimal media suspension. Twelve tubes contained Pb free Fe-oxyhydroxides and were also inoculated with microbe/media suspension. Controls consisted of 12 tubes containing Pb bearing Fe-oxyhydroxide in minimal media and 4 tubes containing only bacteria and minimal media. The anaerobic media suspension was added to
all reaction vessels in an anaerobic chamber (Coy Laboratories Inc., Grass Lake, MI, N2:H2 (96:4)). The reaction vessels were rotated end-over-end at 30 rpm and incubated at 30°C in the absence of sunlight.

2.2.4 Sampling strategy

Batch sampling was conducted by sacrificing selected vessels periodically over a period ranging from 6 hrs to 2 months (Figure 4). Each sample was removed from the agitating wheel and an aliquot of slurry was removed for the determination of extractable Fe(II) and for cell counting. After resealing the sample tubes and wrapping them with parafilm to minimize air contamination the tubes were removed from the anaerobic chamber and centrifuged at 4000 g for 5 minutes. The tubes were then returned to the anaerobic chamber where the liquid was removed using silicon tubing and a 60 ml syringe. The supernatant was filtered through a 0.45 um syringe (Fisher non-sterile nylon) directly into another 60 ml syringe connected to the filter via silicon tubing. This again was to minimized exposure to the chamber atmosphere that may have contained traces of oxygen (Jeon et al., 2004). The filtrate was then separated into several vessels for the various aqueous analyses: 5-10 ml for alkalinity and Fe(II) measurements; 5 ml for the determination of Pb by ICP-MS (acidified to 1% v/v using sub-boiling distilled HNO3); and 5 ml for Eh and pH measurements. The remaining filtrate (approximately 25-30 ml) was reserved for voltammetric analysis and was kept in the syringe; the tip of which was thoroughly wrapped with parafilm. The syringe
and acidified ICP-MS samples were removed from the anaerobic chamber and stored at -80°C.

2.2.4.1 Aqueous chemistry

Determinations of Fe(II) (Viollier et al., 2000) and alkalinity (Sarazin et al., 1999) were conducted within the anaerobic chamber using a ThermoSpectronic model Genesys 20 visible light spectrophotometer. Eh and pH were determined using combination electrodes (VWR sympHony probe) and a pH/Eh meter (VWR sympHony SR301) which was calibrated using 3 NIST standards immediately before all measurements. Samples were acidified and diluted with (sub-boiling doubly distilled) Nitric Acid for Pb analysis by ICP-MS. An internal standard (Tl) was used to correct for instrumental drift and mass bias.

2.2.4.2 Solid Phase characterization

The solid material that was left after the centrifugation was freeze dried immediately and then stored under N₂ in hungate tubes to prevent further oxidation or phase changes. Solid samples for XRD and BET-surface area analyses were prepared by grinding into a fine powder using an agate mortar and pestle and acetone. Grinding the sample is necessary for the XRD preparation though it will not influence the surface area measurements (Vucelic et al., 2002). After the XRD and BET data was collected the entire sample (30-200 mg) was used for solid phase selective sequential extractions (Poulton and Canfield, 2005) to determine both the phase composition of the solid phases and the distribution of Pb within
these phases. To target specific mineral phases, the following solid phase selective sequential extractions (POULTON and CANFIELD, 2005) were employed:

1. Sodium acetate:
   These included 1 M sodium acetate adjusted (with acetic acid) to pH 4.5 at room temperature.

2. Hydroxylamine–HCl:
   A 1 M hydroxylamine–HCl solution in 25% v/v acetic acid, suitability as an extractant of ‘easily reducible’ oxides (ferrithydrate and lepidicrocite).

3. Sodium(Citrite) dithionite:
   A sodium dithionite solution (50 g l⁻¹) buffered to pH 4.8 with 0.35 M acetic acid/0.2 M sodium citrate for the selective extraction of ‘reducible’ oxides (goethite, hematite and akaganeite). This extraction was applied for 2 hr.

4. Ammonium oxalate:
   A 6 hr 0.2 M ammonium oxalate/0.17 M oxalic acid solution (pH 3.2) suitable as an extractant of magnetite was applied.

5. 6N HCl:
   A 6N HCl extraction suitable for Fe-(oxyhydr)oxides, siderite and ankerite, in addition to some Fe from certain sheet silicate minerals (e.g. nontronite, chlorite, glauconite, biotite) was applied for 96hrs.

The extractant was diluted and Fe and Pb concentrations were determined by ICP-OES.
2.2.4.3 Thermodynamic equilibrium Calculations

Thermodynamic equilibrium calculations were made using the Jchess geochemical modeling code and chess thermodynamic database. Activity coefficients were calculated using the truncated Davis equation. Thermodynamic sorption reactions were calculated using the default surface complexation reaction law and the double layer model. Calculations were made for a pH of 6.8, temperature of 25°C, an Eh of 0 volts, HCO₃⁻ concentration of 2 mM, a Pb concentration of 33.45 uM and a hydrous ferric oxide concentration of 50 mM. The hydrous ferric oxide was assigned a surface area of 160 m² g⁻¹.

2.3 Results and Discussion

2.3.1 Fe-oxyhydroxide characterization

The oxyhydroxide produced by controlled hydrolysis during the titration with KOH, has a molar lead substitution of Pb:Fe $6.67 \times 10^{-4}$ M as determined by ICP-MS via acid digestion. The pH was monitored during the titration and is plotted in Figure 5, as a function of base added. The nearly identical form of these plots for both Pb-Free Fe-oxyhydroxides (herein referred to as Fe-OH) and Pb substituted Fe-oxyhydroxide (Pb-Fe-OH) titrations provides some confidence that both were synthesized under similar conditions. During titration, the aqueous iron concentration decreased rapidly at pH ~ 4.5 and simultaneously, Pb was removed from solution. XRD spectra of both Fe-OH and Pb-Fe-OH are indistinguishable, and are characteristic of a mixture of poorly crystalline ferrihydrite and the more crystalline mineral akaganeite (Figure 3) (SCHWERTMANN and CORNELL, 1996). All
XRD were taken using a Miniflex XRD, acquiring XRD spectra in the range of 5 to 90 deg with 0.1 deg angle resolution and acquisition time of 1 min per interval, resulting in a total acquisition time of approximate 7 hrs per sample. Specific surface areas (Table 2) coincide with the XRD results, as all hydroxides precipitated have higher (80-170 m² g⁻¹) values characteristic of amorphous minerals. These surface areas were measured using a five point (N₂ sorption) BET (Quantachrome Nova 3000) analysis, degassing the sample at room temperature (21°C) for 24 hrs prior to analysis.

2.3.2 Iron reduction

Aqueous Fe (II) (Fe(II)aq), in both Pb-bearing Fe-oxyhydroxide incubation (Pb-Fe-OH-I) and Pb-free Fe-oxyhydroxide incubations (Fe-OH-I), increased rapidly until it reached a plateau at 1,700 μmol l⁻¹ and 1,200 μmol l⁻¹, respectively, after approximately 200 hrs (Figure 6). The Fe(II)aq concentrations in the sterile controls remained below the detection limits of the ferrozine method throughout the experiment. Although initially lower, the Fe(II)aq concentration in the Pb-Fe-OH-I samples became greater than that in Fe-OH-I. The 0.5 M HCl extractible Fe(II) (ie Fe(II)ex) concentrations were the same after 30 hrs in both the Pb-Fe-OH-I and Fe-OH-I samples (Figure 7).

The quantity of Fe(II)ex is a good proxy for the extent of metabolic activity (RODEN, 2003; RODEN and ZACHARA, 1996). As Fe(II)ex was equivalent for both Pb-Fe-OH-I and Fe-OH-I, and pH and alkalinity generation remained similar throughout the experiment, it can be deduced that both have a similar reactivity.
and metabolic activity. As such, the Pb substitution at a molar concentration of Pb:Fe $6.67 \times 10^{-4}$ did not impede the metabolic activity of S. putrefaciens 200R; via a toxic effect or surface passivation over the duration of this experiment. Therefore, the difference in Fe(II)$_{aq}$ between Fe-OH-I and Pb-Fe-OH-I demonstrates that the presence of Pb in Fe-oxyhydroxides inhibits Fe(II) removal from solution.

Rate constants were calculated using first order reaction kinetics to approximate the rate of release of Pb and Fe. For the Fe-OH-I system, Fe(II)$_{aq}$ and Fe(II)$_{ex}$ were released at a rate of $7.68 \times 10^{-13}$ and $1.88 \times 10^{-12}$ mol m$^{-2}$ min$^{-1}$, respectively. In the case of Pb-Fe-OH-I, Fe(II)$_{aq}$ was released at a rate of $3.46 \times 10^{-12}$ mol m$^{-2}$ min$^{-1}$ and Fe(II)$_{ex}$ at $1.45 \times 10^{-12}$ mol m$^{-2}$ min$^{-1}$. These values are lower than previously reported studies with the same biomass ($2.5 \times 10^{-9}$ mol m$^{-2}$ min$^{-1}$) (RODEN, 2003), however, detailed information regarding the minimal media composition is lacking, which may explain some of the discrepancy between the results.

2.3.3 Secondary Mineralization

Secondary mineralization was evident after 12 hours of incubation due to changes in the colour of the suspensions, from the deep red characteristic of akaganeite, to black (pure oxyhydroxide) and mustard (Pb substituted oxyhydroxide) hues (SCHWERTMANN and CORNELL, 1996). XRD analysis confirmed the precipitation of goethite in the incubations containing Pb (Figure 8). The intensity of the peaks, measured through XRD, characteristic of goethite, increased...
as a function of time for Pb-Fe-OH-I (Figure 9). This suggests progressive goethite
growth throughout the duration of the experiment. In contrast, XRD patterns of Fe-
OH-I show peaks characteristic of magnetite (Figure 10). The magnetite peaks
also increase in intensity over time suggesting magnetite growth (Figure 11). The
control, Fe-OH, did not demonstrate any evidence of secondary mineralization, as
the XRD spectra remained constant over time (Figure 12).

The growth of magnetite in these experiments would be expected, based
upon previous work with poorly crystalline Fe-oxyhydroxides (COOPER et al., 2000;
LOVELY et al., 1987; STOLZ et al., 1990; ZACHARA et al., 2002). In addition,
thermodynamic equilibrium calculations yield saturation indices, that are >0 for
magnetite (Table 3). These Saturation indices are defined as: $S_i = \log(I_{ap}/K_{sp})$
where $S_i$ is the Saturation index, $I_{ap}$ is the ion activity product and $K_{sp}$ is the
solubility product for a given mineral phase. Therefore, Saturation indexes >0
indicate that the system is supersaturated with respect to magnetite.

The predominance of goethite in the Pb-Fe-OH-I was surprising, and
suggests that the presence of low concentrations of Pb inhibits magnetite
formation. This inhibition of magnetite growth in Pb-Fe-OH-I is likely the combined
result of high concentrations of inner-sphere surface adsorbed Pb and
incompatibility of this Pb in the magnetite structure; thereby, preventing the
nucleation of surface magnetite. Pardo et al, 2002, alluded to the incompatibility of
Pb into the magnetic structure. Ford et al, 1997, found that Pb was not
incorporated into the goethite structure as other metals were, thus affecting the
behaviour of Pb in solution. The experiments of Cooper et al. (COOPER et al.,
2005; COOPER et al., 2000; COOPER et al., 2006), in which Fe-oxyhydroxides were incubated with Zn, yielded magnetite as a secondary mineral. Indeed, the incorporation of Zn into secondary magnetite was invoked as an important mechanism in the sequestration of Zn under Fe reducing conditions (COOPER et al., 2005; COOPER et al., 2000; COOPER et al., 2006).

2.3.4 Lead Mobilization

The concentration of dissolved Pb in solution with Pb-Fe-OH-I was found to increase over time (Figure 13). The dissolved Pb remained below 1 nmol l\(^{-1}\) in Pb-Fe-OH (control) (Figure 13). This demonstrates that Pb release was a function of DIR. The rate of Pb release was calculated using first order rate kinetics, and was found to be in the range of \(1.09 \times 10^{-17}\) mol m\(^{-2}\) min\(^{-1}\) and \(2.56 \times 10^{-17}\) mol m\(^{-2}\) min\(^{-1}\), amounting to a concentration of 192 nmol l\(^{-1}\) or approximately 0.6% of the total Pb at the end of the experiment. The final concentration of Pb in the sample at the end of the experiment was highly consistent with calculated values of 195.3 nmmol l\(^{-1}\) based on the Thermodynamic equilibrium equations. Thermodynamic equilibrium calculations predict that the dominant aqueous Pb species is PbCO\(_3\)\(^{\text{aq}}\). PbCO\(_3\)\(^{\text{aq}}\) comprised 56% of the total dissolved Pb, whereas the free Pb ion only comprised 39%. The remaining 5% is comprised of various ionic Pb hydroxyl species. The dissolution of Pb was less than congruent, but approached congruency towards the end of the experiment (Figure 14). This indicates that the solid phase products of dissimilatory Fe reduction became enriched in Pb with respect to the initial Fe-oxyhydroxides.
2.3.5 Solid Phase extractions

The first extraction (Sodium Acetate) dissolves carbonate minerals and then the following, (Hydroxylamine-HCL, Citrite Dithionite, Ammonium Oxalate and 6N HCL), progressively dissolve 'easily reducible' oxyhydroxides, 'reducible' Fe-oxyhydroxides and finally more refractory Fe-oxides and silicates.

For the control (Pb-Fe-OH) sample, all of the extractable Fe and Pb were extracted in the first three steps (Sodium Acetate, Hydroxylamine-HCL, Citrite Dithionite), and the release of Fe and Pb did not vary over time. The majority of extractable Fe was released in the Hydroxylamine-HCL extraction (~70% by mass), and Pb release was evenly distributed between the Sodium Acetate and Hydroxylamine-HCL. As release rates remained constant over time, it seems that there were no apparent transformations occurring in the control samples.

The Fe-OH-I and Pb-Fe-OH-I samples had three major trends (Figure 15 & Figure 16). As expected, based on the use of a low carbonate, HEPES buffered minimal media composition (Table 1.), there was very little Sodium Acetate extractable Fe. The Fe-carbonate formation was minimal over the duration of the incubations and is consistent with the absence of siderite peaks in the XRD patterns (Figure 10). Phases defined as "easily reducible" (i.e. ferrihydrite and lepidocrocite) that are dissolved in the Hydroxylamine-HCL extraction, were present in relatively high concentrations (~50% by mass) in the starting materials and then quickly dropped with the onset of Fe reduction to a constant level around 10%. Phases defined as "reducible oxides" (i.e. goethite, hematite and akaganeite) were abundant (40-50%) in the starting materials. The "reducible
oxides" content of the Fe-OH-I system remained approximately equivalent throughout the duration of the incubations. However, the "reducible oxides" content of the Pb-Fe-OH-I system decreased rapidly to between 10% and 20%. The ammonium oxalate extractable fraction (which is largely magnetite) was <5% in the starting material, but rapidly increased to 40% after about 120 hrs of incubation in the Fe-OH-I system, and remained below 10% in the Pb-Fe-OH-I system. These observations are consistent with the XRD patterns (Figure 8 & 10) and clearly demonstrate that the presence of Pb inhibits crystallization to magnetite. The 6N HCl extractable fraction in the Fe-OH-I, which should include even the most refractory of Fe oxides, decreased rapidly from 10% to <5% and then stayed relatively constant. In contrast, the 6N-HCl extractable fraction in the Pb bearing systems increased rapidly to nearly 80% of the total Fe pool.

The difference in mineralization pathways between the Pb bearing and Pb free systems has several important implications. For example, the inhibition of magnetite formation in the presence of Pb may contribute to the paucity of magnetite in zones of Fe-reduction in aquatic sediments. Simple experiments with monomineralic and non trace element bearing Fe-oxyhydroxides frequently predict that magnetite is that dominant solid phase product during DIR and is a significant sink for Fe(II) produced during DIR. However, such authigenic magnetite formation is rarely observed and can be difficult to detect in the environment. This may be, in part, due to the trace element (including Pb) rich nature of natural Fe oxyhydroxides. This inhibition of magnetite formation should be considered when interpreting rock magnetic data that is frequently used to infer information about
the redox state of modern and paleo-sediments (Reitz et al., 2004). Magnetite has also been suggested as an important sink for Zn under Fe reducing conditions (Cooper et al., 2000). Therefore, Zn mobility and residence times may be enhanced in the concomitant presence of Pb. In addition, the formation of a refractory Fe phase, only extractable by 6N HCl, in the Pb bearing systems suggests that this Fe may be less reactive to subsequent transformations such as the formation of Fe sulphides. The identity of this poorly reactive phase remains unknown, and the effect of trace metal substitutions on Fe cycling and residence times should be further investigated.

The sequential extractions used to operationally identify different Fe-phases can also be used to evaluate the partitioning of Pb in these phases. Data for Pb released during these extractions (Figure 17) show that the most of the Pb was bound to surface reactive phases (Sodium Acetate extractable). One possibility is that the Pb could have been bound by sorption or other weak binding mechanisms to the Fe mineral surfaces. Alternatively, as the Acetate extraction is designed to dissolve carbonate phases, the Pb released from Fe-oxyhydroxides during the incubations may have precipitated as Pb carbonates, since there is a significant alkalinity ($CO_3^{2-}$) present averaging approximately 10 mM, which did not change over the course of the experiment. This hypothesis is consistent with the fact that equilibrium calculations predict that hydrocerussite ($Pb_3(CO_3)_2(OH)_2$) and cerussite ($PbCO_3$) were supersaturated in the system (Table 3). Therefore, the Pb may have existed in the form of Pb-carbonate, or could have been bound by sorption or other weak binding mechanisms to the mineral surfaces. The rapid decrease of Pb in the
Hydroxylamine-HCL extractible fraction suggests Pb was redistributed to non-redducible phases during Fe reduction. All the other phases remained negligible from time 0, with the exception of the 6N HCL extractible fraction which increased slightly over time, implying that Pb is associating with more unreactive phases. Thus, despite the fact that Pb is partly mobile (~10%) during DIR, it is also removed from reducible fractions (to ~10%) and incorporated into both a highly reactive solid fraction (~50%) and very refractory solid fraction (~30%).
2.4 References


Table 1. Composition of media utilized in the experimental systems.

<table>
<thead>
<tr>
<th>Component</th>
<th>MW</th>
<th>Concentration (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCl</td>
<td>74.56</td>
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</tr>
<tr>
<td>NH₄Cl</td>
<td>53.49</td>
<td>28</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>147.01</td>
<td>0.68</td>
</tr>
<tr>
<td>NaClO₄</td>
<td>140.46</td>
<td>50</td>
</tr>
<tr>
<td>Lactate</td>
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<td>24</td>
</tr>
<tr>
<td>HEPES</td>
<td>238.3</td>
<td>30</td>
</tr>
</tbody>
</table>

Table 2. Multipoint-BET surface area measurements of the samples utilized in the experimental system. Typical values for synthetic production of minerals provided for reference (SCHWERTMANN and CORNELL, 1996).

<table>
<thead>
<tr>
<th></th>
<th>Initial Surface Area</th>
<th>Final Surface Area</th>
<th>Average</th>
</tr>
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<tbody>
<tr>
<td>Pb-bearing Fe-oxyhydroxide</td>
<td>168 m² g⁻¹</td>
<td>79 m² g⁻¹</td>
<td>111 m² g⁻¹</td>
</tr>
<tr>
<td>Pb-Free Fe-oxyhydroxide</td>
<td>106 m² g⁻¹</td>
<td>46 m² g⁻¹</td>
<td>79 m² g⁻¹</td>
</tr>
<tr>
<td><strong>Typical Value for Synthetic Minerals</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goethite</td>
<td></td>
<td>80 m² g⁻¹</td>
<td></td>
</tr>
<tr>
<td>Magnetite</td>
<td></td>
<td>4 m² g⁻¹</td>
<td></td>
</tr>
<tr>
<td>Akaganeite</td>
<td></td>
<td>100 m² g⁻¹</td>
<td></td>
</tr>
<tr>
<td>Ferrihydrite</td>
<td></td>
<td>200 m² g⁻¹</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Saturation Index results when precipitation in the system is disabled, using a truncated-davies surface-complexation model.

<table>
<thead>
<tr>
<th>Mineral Solid</th>
<th>Saturation Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrocerussite</td>
<td>4.889</td>
</tr>
<tr>
<td>Hematite</td>
<td>4.167</td>
</tr>
<tr>
<td>Cerussite</td>
<td>2.243</td>
</tr>
<tr>
<td>Magnetite</td>
<td>2.152</td>
</tr>
<tr>
<td>Goethite</td>
<td>1.603</td>
</tr>
<tr>
<td>Ice</td>
<td>-0.1387</td>
</tr>
<tr>
<td>Siderite</td>
<td>-1.095</td>
</tr>
<tr>
<td>PbCO₃·PbO</td>
<td>1.968</td>
</tr>
</tbody>
</table>
Figure 3: Initial Fe-hydroxide mineral compared to Akaganeite Standard
Figure 4: Sampling protocol utilized for the aqueous and solid phase.
Figure 5: Base titration data from the synthesis of Pb-bearing and Pb-Free Fe-oxyhydroxide minerals.
Figure 6: Aqueous Fe(II) release as a function of time for Pb bearing mineral inoculations (Pb-Fe-OH-I), Pb free mineral inoculations (Fe-OH-I) and Controls [Pb bearing mineral not inoculated(Pb-Fe-OH)].
Figure 7: 0.5M HCL extractible Fe(II) as a function of time for Pb bearing mineral inoculations (Pb-Fe-OH-I) and Pb-Free mineral inoculations (Fe-OH-I).
Figure 8: Pb-Bearing inoculated Fe-oxyhydroxide XRD spectra compared to goethite standard.
Figure 9: XRD Spectra over time for the Pb-bearing inoculated Fe-oxyhydroxide sample.
Figure 10: Pb-Free inoculated Fe-oxyhydroxide XRD spectra compared to magnetite and goethite standards.
Figure 11

Figure 11: XRD spectra over time for the Pb-Free inoculated Fe-oxyhydroxide sample.
Figure 12: Spectra over time for the Pb-free non-inoculated Fe-oxyhydroxide control
Figure 13: Aqueous Pb release as a function of time for inoculated (Pb-Fe-OH-I) and un-inoculated (Pb-Fe-OH) Pb bearing Fe-oxyhydroxides
Figure 14: Congruency plot of Pb in presence of Fe.
Figure 15: Fe release as a function of time by solid phase sequential extraction of the inoculated Pb-Free Fe-oxyhydroxide mineral (Fe-OH-I).

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Figure 16: Fe release as a function of time by solid phase sequential extraction of the inoculated Pb bearing Fe-oxyhydroxide mineral (Pb-Fe-OH-I).
Figure 17: Pb release as a function of time by solid phase sequential extraction of the inoculated Pb bearing Fe-oxyhydroxide mineral (Pb-Fe-OH-I).
CHAPTER 3 CONCLUSIONS

3.1 Conclusion

During Dissimilatory Iron Reduction (DIR), up to 190 nmol t\(^{-1}\) Pb was released into the aqueous phase at a rate of 1.82x10\(^{-17}\) mol m\(^{-2}\) min\(^{-1}\) from Pb-bearing Fe-oxyhydroxides. The presence of such high concentrations of Pb in solution is inconsistent with the fact that 94.8% of the Fe-oxyhydroxide remains undissolved. The presence of copious amounts of residual Fe-oxyhydroxides, that exhibit high specific surface (≈140 m\(^2\) g\(^{-1}\)) areas, should limit the aqueous Pb concentrations to low levels. This suggests that Pb is stabilized in solution by the presence of ligands possessing a high affinity for Pb and forming soluble complexes. These may be Pb specific moieties like soluble components of EPS or ligands specific for metals like Pb (Guibaud et al., 2005). Such a scenario has been invoked to explain the coexistence of high concentrations of Fe-oxides and high concentrations of aqueous Pb proximal to roots in salt marshes (SUNDBY et al., 2005). This raises interesting questions about models of sorption and bioavailability of Pb and other trace metals in natural systems which typically presume the major aqueous reservoir of Pb in these settings are the free ion.

Pb concentrations in the Fe-oxyhydroxides used in this experiment, of 6.67x10\(^{-4}\) Pb:Fe ratio in the Fe-oxyhydroxide are not uncommon in natural settings (SUNDBY et al., 2005). The Pb concentration required to induce toxic effects in S. putrefaciens 200R is unknown, however in this study the Pb present in the Fe-oxyhydroxides does not inhibit respiration. This conclusion has been drawn from the similar Fe-reduction rates of the regular and Pb bearing Fe-oxyhydroxide.
Finally, it appears that the presence of Pb inhibits the Fe-oxyhydroxide recrystallization to magnetite that is commonly observed during DIR. This is evident from both XRD spectra as well as from sequential selective extractions. The presence of Pb appears instead to favor recrystallization to small quantities of goethite and an unknown refractory phase only soluble in 6N HCl. The fact that natural Fe-oxyhydroxides are rich in trace elements including Pb may explain why in many natural environments there is minimal to undetectable magnetite formation during DIR, even though laboratory studies using pure Fe-oxyhydroxides predict magnetite to be the dominant secondary solid phase forming.

3.2 Future direction and research

In order to make this dataset more complete, I would like to add a visualization component to the project that would be comprised of a series of SEM images of the samples to aid in the identification of mineral structures and possible microbial mineral interactions. Pseudovoltammetry (ROZAN et al., 2003) could improve the quality and quantity of my interpretation of the aqueous data and may prove invaluable in explaining the large amounts of Pb that remain in solution even at a supersaturated state; hopefully it will also reveal speciation of Pb and Fe(II) in solution as well as identify types of ligands present. Analysis for bacterial Extra Polymeric Substances (EPS) would also be highly topical in this context. To further evaluate the surface binding sites and structure of the secondary minerals, synchrotron based X-ray Adsorption Fine-structure Spectroscopy (XAFS) would be an appropriate method to undertake. Altering parameters such as Pb
concentration, mineral composition, bacterial strain, and even concentrations of other metals would provide further insight into the interactions of trace metals in the environment. The further examination of these processes on $^{210}\text{Pb}$ mobility should also be examined in greater detail, so that a stronger basis for these types of dating methods can be established.
3.3 References


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