LIU, YU-TING. Phosphate Sorption and Reductive Dissolution in Aluminum/Iron-Hydroxide Co-Precipitates. (Under the direction of Dr. Dean Hesterberg).

Poorly-crystalline Al(III)- and Fe(III)-hydroxides have a high capacity to sorb orthophosphate (PO$_4$). Due to the redox-active characteristic of Fe, fluctuation in redox potential might be one of the major factors influencing PO$_4$ fate in soils and sediments. The goal of this research was to determine molecular mechanisms of PO$_4$ retention and dissolution in non-crystalline Al(III)/Fe(III)-hydroxide co-precipitate systems as affected by dissimilatory microbial Fe(III) reduction. The specific objectives were: (1) to quantify the relative distribution of sorbed PO$_4$ bonding between Al(III) and Fe(III) in relation to the structural properties of Al/Fe-hydroxide co-precipitates; (2) to determine how Al co-precipitation affects Fe(III) bioreducibility in relation to the local coordination structure and transformations in Fe-hydroxide domains; and (3) to characterize PO$_4$ re-distribution between solid phases and aqueous solutions during bioreduction of Al/Fe-hydroxide co-precipitates.

Phosphate sorption isotherms experiments at PO$_4$ concentrations between 42 and 162 mmol mol$^{-1}$ Al+Fe were conducted on Al/Fe-hydroxide co-precipitates with Al/(Al+Fe) molar ratios of 0, 20, 50, 75, and 100 mol%. Characterization using x-ray absorption near edge structure (XANES) spectroscopy shows preferential PO$_4$ bonding to Al on co-precipitates containing $\leq$ 50 Al mol%, but a non-preferential PO$_4$ distribution to either Al or Fe as Al proportion was increased to 75 mol%. Such PO$_4$ distribution was correlated to the near-surface composition of Al as indicated by x-ray photoelectron spectroscopy (XPS). Along with increasing proportion of co-precipitated Al, trends in decreasing structural ordering and
decreasing size of Fe domains were found. After 168 h of bioreduction promoted by *Shewanella putrefaciens* CN32, total Fe(II) production increased 40-fold as co-precipitated Al increased from 0 to 50 mol%. Over the course of bioreduction, Al tended to impede microbial-induced structural development in Fe domains. Apparently by optimizing surface interactions between bacterial cells and Fe(III)-hydroxides, increasing Al enhanced electron transfer between Fe(III) and microbes. Alternatively, bioreduction of phosphated Al/Fe-hydroxide co-precipitates was not only a function of Al proportion, but also a function of sorbed PO₄ concentration. In co-precipitates with ≤ 20 mol% of Al, the Fe(III) bioreducibility increased with increasing sorbed PO₄ concentration. Analogous to co-precipitated Al, PO₄ stabilized Fe domains against polymerization. For samples with ≥ 50 mol% of Al, however, effects of Al-hydroxide appeared to be greater than that of PO₄, resulting in no effect of sorbed PO₄ concentration on Fe(III) bioreducibility. During the 168-h bioreduction period, no reductive dissolution of PO₄ was detected, contrary to my hypothesis. Availability of Al and Fe binding in residual solids during bioreduction accounted for PO₄ retention. Accompanying enhanced Fe(III) bioreduction, an increase in Fe[(II)/(III)]-PO₄ bonding was generally responsible for PO₄ sorption in systems with Al ≤ 20 mol%. As Al(III) became dominated (Al ≥ 50 mol%), however, a preferential PO₄ bonding to Al was found in residual solids. Superior PO₄ sorption capacity of Fe[(II)/(III)] and Al(III) polymers retained PO₄ against other possible solid-phase retention pathways such as precipitation of biominerals or microbial uptake, and thereby constrained reductive PO₄ dissolution. In general, my research showed that enhanced dissolution of PO₄ observed under reducing soil conditions depends on processes other than direct release of PO₄ into solution during reductive dissolution of Fe(III)-hydr(oxide) minerals.
Phosphate Sorption and Reductive Dissolution in Aluminum/Iron-Hydroxide Co-Precipitates

by
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DEDICATION

For my parents
BIOGRAPHY

Yu-Ting Liu was born in Taipei, Taiwan. She received her bachelor and master degrees in Agricultural Chemistry from National Taiwan University (Taipei, Taiwan) in 2003 and 2005. After graduating, she worked as a research assistant at the lab of soil environmental chemistry at National Taiwan University for one year. During this period, she confirmed her interest in scientific research pertaining to environmental chemistry. In August 2006, she enrolled in North Carolina State University to start her doctoral degree with the major of soil chemistry in department of Soil Science under the guidance of Dr. Dean Hesterberg. Her goal is to deliver the passion of science via being a faculty member in a college.
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CHAPTER 1
General Introduction

Background

Phosphorus (P) in soils occurs almost exclusively in the form of phosphate (PO$_4$) (Mengel and Kirkby, 2001). In past few decades, excess PO$_4$ due to the over application of fertilizers has been recognized throughout the world as an agricultural pollutant. If a large amount of PO$_4$ is applied to soil, it may move to surface waters with runoff and eroded soil particles that bind PO$_4$. The major consequence is a process called eutrophication. In 1996, the USEPA reported that more than 50% of freshwater eutrophication is attributed to agricultural nutrients such as P (U.S. Environmental Protection Agency, 1996). Variable abiotic and biotic factors, such as pH, redox potential, organic matter content, competitive adsorbates, nonreductive/reductive dissolution of adsorbents, and microorganisms, have been considered to greatly affect the reactivity, speciation, mobility, and bioavailability of PO$_4$. Natural PO$_4$ cycling could be assigned to inorganic or biological processes. Inorganic processes include physicochemical reactions, such as precipitation/dissolution and sorption/desorption that related to the presence of soil minerals.

Phosphate Retention on Al- and Fe-(Hydr)oxides

Phosphate sorption maxima of common minerals or non-crystalline solids in soils have been reported at 3400, 1900, 280, 158, 70, and 15.2 mmol kg$^{-1}$ for non-crystalline Al-hydroxide, ferrihydrite, gibbsite, goethite, hematite, and kaolinite, respectively (Dimirkou et
al., 2002; Khare et al., 2005; Murray and Hesterberg, 2006; Violante and Pigna, 2002). Apparently, non- and poorly-crystalline Al- and Fe-(hydr)oxides provide the major PO$_4$ retention capacity in soils. This statement agrees with that reported by Beauchemin and Simard (1999): PO$_4$ sorption capacity in soils has been related to the indices based on acid-oxalate extractable Al and Fe, suggesting that poorly-crystalline Al- and Fe-(hydr)oxides are largely responsible for PO$_4$ retention in acidic soils.

Ferrihydrite (Fe$_5$HO$_8$·4H$_2$O), a common product of low-temperature geochemical processes at the earth’s surface and a general form of Fe(III)-hydroxides in soils and sediments undergoing fluctuating redox conditions (Cornell and Schwertmann, 2003), was chosen as the target PO$_4$ adsorbent in this study. Superior PO$_4$ sorption capacity and redox-active characteristic of ferrihydrite may potentially influence agrogeochemical cycles of PO$_4$ (Parfitt, 1989; Willett et al., 1988). The PO$_4$ adsorption behavior, including bonding mechanisms and protonation of surface complexes, at the ferrihydrite-water interface was investigated using attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy by Arai and Sparks (2001). They suggested the formation of inner-sphere surface complexes between PO$_4$ and ferrihydrite, which are nonprotonated, bidentate binuclear species at pH $\geq$ 7.5 and might co-exist with different surface species such as monodentate mononuclear complexes. However, ATR-FTIR failed to determine the exact identity of the protonated PO$_4$ inner-sphere complexes forming at pH <7.5. This deficiency could be partially overcome by the techniques of P K-edge x-ray absorption near edge structure (XANES). Via XANES, the bidentate-binuclear surface complexes were implied as the predominant mechanisms for PO$_4$ adsorbed on ferrihydrite at pH 6.0 (Khare et al., 2007).
However, surface precipitation was proposed as the major configuration between PO$_4$ and Al-(hydr)oxides of boehmite and non-crystalline Al-hydroxide at pH 6.0 (Khare et al., 2005).

In contrast to the featureless pre-white line region of P K-edge XANES spectrum for variscite (AlPO$_4$·2H$_2$O), a pre-edge peak was found at -3 eV (relative energy) in the spectrum for strengite (FePO$_4$·2H$_2$O) (Hesterberg et al., 1999). This observation proposed the potential of P K-edge XANES spectroscopy to differentiate species of Fe-bound PO$_4$ from Al-bound PO$_4$. Determined using the projected density of states (PDOS), the pronounced pre-white line feature for Fe-bound PO$_4$ is due to the well separated Fe-O and P-O antibonding states in energy, and the overlapping energies between the Al-O and P-O antibonding states causes a less discernible pre-white region for Al-PO$_4$ (Khare et al., 2007). Therefore, P K-edge XANES is able to differentiate relative PO$_4$ distribution on Al- vs. Fe-(hydr)oxides. Khare et al. (2004) used linear combination fitting (LCF) across the pre-white line region of XANES spectra to determine the relative association of adsorbed PO$_4$ between Al and Fe in mixtures of boehmite and ferrihydrite, and found out that the PO$_4$ distribution is a function of adsorbed PO$_4$ concentration. At an adsorbed phosphate concentration of 100 mmol kg$^{-1}$, ferrihydrite showed a great affinity to bond PO$_4$, but no apparent preference to either mineral was observed at concentrations between 200-600 mmol kg$^{-1}$. Alternatively, a preferential PO$_4$ bonding to Al occurred while adsorbed PO$_4$ concentration increased up to 1300 mmol kg$^{-1}$. In fact, the distribution of sorbed PO$_4$ varied with various combinations of Al- and Fe-oxide minerals. In mixtures of goethite/boehmte and ferrihydrite/non-crystalline Al-hydroxide, the preferential PO$_4$ bonding to Al generally dominated PO$_4$ retention behaviors (Khare et al., 2005).
Moreover, features in XANES spectra were sensitive to the geometric configuration of PO₄ associated with oxide minerals, making it capable of distinguishing adsorption from precipitation. Compared to the spectra for adsorbed PO₄ species, relatively broader full width at half-maximum height of the white-line peaks in P K-edge XANES spectra provided indication for precipitated PO₄ in Al-hydroxide minerals but not in Fe-hydroxide minerals (Khare et al., 2005). In the mixtures of Al- and Fe-oxide minerals, XANES spectra showed evidence for PO₄ precipitation in goethite/boehmite and ferrihydrite/boehmite mixtures; however, such precipitation was not found in ferrihydrite/non-crystalline Al-hydroxide systems (Khare et al., 2005; Khare et al., 2004).

**Phosphate Retention on Al/Fe-Hydroxide Co-Precipitates**

Soil particles are formed in weathering environments that may produce complex assemblages of PO₄, Al, Fe, and organic matter. In such particles, Al and Fe can serve as bridging metal centers between PO₄ and negatively charged organic functional groups as a PO₄ retention mechanism on organic matter (Hesterberg, 2010). Therefore, the presence of co-precipitated metal-(hydr)oxides should be considered as one of the major components in soils affecting the biogeochemical cycles of PO₄. Particularly, PO₄ retention mechanisms are potentially altered by mineral interactions (Khare et al., 2005), which may cause changes in particle size distribution, surface charge, and adsorption characteristics of mixed (hydr)oxides (Anderson and Benjamin, 1990). In binary mixtures that contained Al- and Fe-hydroxides, Al-enriched particle surfaces suggested that Al-hydroxides played an important role in determining surface characteristics in soils, sediments, and aqueous environments.
(Anderson and Benjamin, 1990). Beyond the ample knowledge about PO₄ sorption mechanisms in single-component or physical mixtures of Al- and Fe-(hydr)oxide minerals, an attempt to study PO₄ retention on Al/Fe-hydroxide co-precipitates is limited.

Harvey and Rhue (2008) reported that variation in Al/Fe molar ratio influenced the structural development and anion exchange capacity (AEC) of Al/Fe-hydroxide co-precipitates and subsequently their PO₄ sorption characteristics. Lower structural development and higher AEC with increasing Al proportion enhanced PO₄ sorption. This higher AEC also induced a greater initial sorption rate and a less desorption at higher pH values. However, the increasing Al/Fe molar ratio in co-precipitates synthesized by Masue et al. (2007) induced a decreasing adsorption capacity and bonding strength for arsenate (AsO₄), as a surrogate for PO₄. The lower AsO₄ adsorption in the samples with Al ≥ 50 mol% is partially attributable to the formation of bayerite and gibbsite that decreased the number of surface adsorption sites.

**Phosphate Dissolution under Reducing Conditions**

Soil with lower redox potentials (more reduced conditions) showed enhanced PO₄ dissolution that has been proposed to be related to the reductive dissolution of phosphated Fe-(hydr)oxides (Holford and Patrick, 1981; Patrick and Khalid, 1974; Phillips, 1998; Sallade and Sims, 1997; Willett, 1989). Moreover, PO₄ dissolution might also be induced by the competitive adsorption or formation of aqueous ternary dissolved organic matter (DOM)-Fe-PO₄ or DOM-Al-PO₄ complexes during microbial reduction of phosphorus-enriched Ultisols (Hutchison and Hesterberg, 2004). To better understand the reductive dissolution of
PO$_4$ in soils, research on aqueous systems of ferrihydrite with and without boehmite was conducted in the absence of organic matter and microbes (Murray and Hesterberg, 2006). Here the input of boehmite inhibited reductive dissolution of Fe(III) and PO$_4$, presumably by blocking the electron transfer to surface Fe(III) of ferrihydrite and by forming Al-PO$_4$ complexes. Although Fe-(hydr)oxide minerals may constitute only a small fraction of total solid phases in the environment, these minerals (along with Al-oxides) often control the fate of oxyanions as well as trace metals, and can balance both the pH and the Eh of the associated pore water. The cycling of Fe is controlled by interactions between pore water ingredients as well as the solid phases and is often driven by and coupled with microbial processes. Microbial Fe-(hydr)oxide reduction strongly influences the geochemistry of anaerobic soil and sedimentary environments together with the persistence and mobility of various types of organic and inorganic contaminants in such environments (Lovley, 1991; Lovley, 1995).

**Dissimilatory Fe(III) Reduction**

Dissimilatory reduction of Fe-(hydr)oxides has been documented for a large number of microorganisms including those classified in the *Geobacteraceae* and *Shewanella* families that conserve energy to support growth via anaerobic respiration using Fe(III) as an terminal electron acceptor in a wide range of environments such as soils and aquatic sediments (Lovley, 1997; Lovley et al., 2004). Under the conditions with identical amount and type of electron donor, the rates of this enzymatic reduction are limited by Fe(III)-reducing bacterial cell density and the availability of active surface sites, as controlled by mineral surface areas
and accumulation of surface-bound biogenic Fe(II) (Roden, 2006). Hence, ferrihydrite, compared with other crystalline forms of Fe-(hydr)oxides, is more available as an electron acceptor for dissimilatory metal reducing bacteria (DMRB) mainly because of its larger surface area and greater solubility (Lovley, 1991; Lovley and Phillips, 1986; Zachara et al., 1998). In batch suspensions with DMRB, ferrihydrite is often fully reduced or transformed to secondary Fe(II) containing mineral solids, whereas goethite and hematite are only partially reduced (Fredrickson et al., 2001; Fredrickson et al., 1998; Zachara et al., 1998). Cooper et al. (2005) reported that the key factors influencing the degree of microbial Fe(III) reducibility in sediments are the content of poorly-crystalline Fe-(hydr)oxides.

As the primary source of Fe(III) in subsurfaces, Fe(III)-(hydr)oxides are highly insoluble. Direct contact with Fe(III)-(hydr)oxides is generally considered for dissimilatory Fe(III)-reducing bacteria to deliver electrons (Lovley, 1991). In the case of *Shewanella putrefaciens*, a gram-negative bacterium, sorption of Fe(III)-(hydr)oxides including hematite, goethite, and ferrihydrite takes place within 30 min of exposure to microbes (Glasauer et al., 2001). Such contact between microbes and Fe(III)-(hydr)oxides, however, can be decreased by the addition of Fe(III) chelators or electron shuttles (Lovley, 1997). The soluble chelated Fe(III) is more accessible than insoluble Fe(III)-(hydr)oxides for Fe(III)-reducing microbes. As a facilitator of electron transfer, electron shuttles are able to complete an electron transduction cycle via being reduced by the microbes, diffusing away from the cell, and converting back to the oxidized form after giving electrons to appropriate acceptors like Fe(III)-oxides (O'Loughlin, 2008). The electron shuttles could be endogenous compounds such as quinone, excreted by microbes, or exogenous amendments like humic substances and
9,10-anthraquinone-2,6-disulfonate (AQDS) (Lovley, 1997; Nevin and Lovley, 2002; O’Loughlin, 2008).

**General Objectives**

The goal of this research was to characterize the molecular-scale mechanisms of PO$_4$ retention, migration, and dissolution on assemblages of Al- and Fe-hydroxides under microbial-promoted reduction conditions. To improve the fundamental understanding, this research was conducted in model systems to reveal the interactions among PO$_4$, Al, Fe, and Fe(III)-reducing bacteria. The specific objectives were: (1) to determine the distribution of sorbed PO$_4$ bonding between Al vs. Fe on Al/Fe-hydroxide co-precipitates in relation to the structure properties of non-crystalline Al/Fe-hydroxide co-precipitates (Chapter 2); (2) to characterize the impact of structural changes in Fe domains caused by Al on bioreduction of Al/Fe-hydroxide co-precipitates (Chapter 3); and (3) to determine re-distribution of PO$_4$ between residual solid phases and aqueous solutions during bioreduction of Al/Fe-hydroxide co-precipitates (Chapter 4).
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CHAPTER 2

Phosphate Bonding on Non-Crystalline Al/Fe-Hydroxide Co-Precipitates

ABSTRACT

Poorly-crystalline minerals have high sorption capacities for environmentally important chemical species, but molecular-level mechanisms of sorption on complex mineral assemblages remains largely unknown. We determined the distribution of orthophosphate (PO₄) bonding between Al and Fe in relation to structural properties of Al/Fe-hydroxide co-precipitates. Phosphate was sorbed at concentrations between 42 and 162 mmol P mol⁻¹ Al+Fe on co-precipitates containing 0, 20, 50, 75, or 100 mol% of metal as Al. Phosphorus XANES analyses showed preferential PO₄ bonding to Al on co-precipitates with 20 and 50 mol% Al, and no preference for either metal at 75 mol% Al, consistent with x-ray photoelectron spectroscopy (XPS) analyses of near-surface metal distributions. Structural ordering and the Fe-hydroxide domain size in co-precipitates decreased with increasing Al proportion, as shown by X-ray diffraction (XRD) and Fe EXAFS analyses. Both metal distribution and structure imparted unique sorption properties of co-precipitates compared with isolated metal hydroxides.
INTRODUCTION

Phosphorus is essential for life, but its management in terrestrial environments requires balancing bioavailability and mobility to avoid contamination of water bodies (Daniel et al., 1998; Sharpley et al., 2002). The PO₄ sorption capacity of soils is frequently gauged by acid-oxalate-extractable Al and Fe, indicating that structurally disordered Al- and Fe-(hydr)oxides are dominant sorbents (Beauchemin and Simard, 1999). Such solids are common in soils and waterways (Schwertmann et al., 1982), and their PO₄ sorption capacities are generally greater than those of phyllosilicates and crystalline oxide minerals (Parfitt, 1989; Violante and Pigna, 2002). In soils and other environmental systems, poorly ordered Al- and Fe-(hydr)oxides are likely associated into Al-substituted Fe-(hydr)oxides (Cornell and Schwertmann, 2003; Schwertmann and Taylor, 1989) or complex assemblages of Al- and Fe-hydroxides containing organic matter (Perret et al., 2000). Interactions between dissimilar solid phases and the distribution of sorbed PO₄ between Al- and Fe-(hydr)oxides in complex particles (Harvey and Rhue, 2008; Khare et al., 2005) could be particularly important for PO₄ mobilization under reducing conditions where Fe(III)-(hydr)oxides undergo reductive dissolution (Reddy et al., 1998).

Whereas the distribution of PO₄ between Al and Fe in physical mixtures depended on the crystallinities of the (hydr)oxide minerals involved (Khare et al., 2005; Khare et al., 2004), knowledge about molecular-scale sorption properties of Al/Fe-hydroxide co-precipitates is limited. Harvey and Rhue (2008) reported that PO₄ sorption on poorly-crystalline Al/Fe-hydroxides co-precipitates increased with increasing Al/Fe ratio due to
changes in solid-phase structures and anion exchange capacities. In contrast, Masue et al. (2007) found that sorption of arsenate decreased with increasing Al/Fe molar ratio because of non-crystalline Al-hydroxide transformation to bayerite and gibbsite. Thus, oxyanion sorption on Al/Fe-hydroxide co-precipitates is affected by solid-phase interactions that affect surface functional groups and structural ordering.

The objective of this research was to determine the relative distribution of PO$_4$ bonding between Al and Fe in relation to structural characteristics of Al/Fe-hydroxide co-precipitates. We combined sorption isotherm experiments with spectroscopic analyses that are sensitive to PO$_4$ bonding with Fe vs Al [P K-edge x-ray absorption near edge structure (XANES) spectroscopy] (Khare et al., 2007; Khare et al., 2004) and local coordination structure of Fe-hydroxides [Fe K-edge extended x-ray absorption fine structure (EXAFS) spectroscopy]. Bulk and surface properties of the co-precipitates were also characterized using XRD, XPS, and other techniques.

**MATERIALS AND METHODS**

**Synthesis and Characterization of Non-Crystalline Al/Fe-Hydroxide Co-Precipitates (AFH)**

Samples of AFH were synthesized based on a ferrihydrite synthesis procedure (Schwertmann and Cornell, 2000). Analytical grade Fe(NO$_3$)$_3$·9H$_2$O and Al(NO$_3$)$_3$·9H$_2$O were dissolved into 0.5 L of CO$_2$-free water to achieve a total metal (Al+Fe) concentration of 0.2 mol L$^{-1}$ with Al/(Al+Fe) molar ratios of 0, 0.1, 0.2, 0.5, 0.75, or 1.0 (0-, 10-, 20-, 50-, 75-,
and 100-AFH). Co-precipitation was induced by adjusting the solutions to pH 7.5 with 1.0 M KOH and stirring for 0.5 h (Schwertmann and Cornell, 2000). Precipitates were washed three times each with 1 M KCl solution and 0.01 M KCl by shaking for 30 min and centrifuging (Khare et al., 2004). Final stock suspensions of 0.2 L in 0.01 M KCl solution were adjusted to pH 6.0, characterized for solids concentration (Khare et al., 2004), and stored at 4 °C for a maximum of 2 weeks. A subsample of each suspension was washed with deionized water to remove excess salts, and freeze-dried prior to analyses of total Al and Fe concentration, XRD, XPS, transmission electron microscopy (TEM), Fourier-transform infrared (FTIR) spectroscopy, and specific surface (see Appendix for details).

**Iron K-Edge X-Ray Absorption Spectroscopy (XAS) Data Collection and Analysis**

Local coordination of Fe in AFH samples was characterized using Fe K-edge EXAFS spectroscopy. A moist paste of each AFH samples was collected on a 0.2 µm Millipore polycarbonate filter membrane at a thickness calculated to yield unit edge step across the Fe K-edge near 7112 eV (Kelly et al., 2008). The samples were sealed with 8 µm Kapton film and Kapton tape to avoid desiccation, and stored at 4 °C. Spectra were collected at Beamline X11B at the National Synchrotron Light Source, Brookhaven National Laboratory in Upton, NY. Details for XAS data collection and analyses are given in Appendix.
Phosphate Sorption Isotherms

Phosphate sorption isotherms for AFH samples were determined at pH 6.0 and 25 °C in a 0.01 M KCl background. Each sample contained 30.00±0.05 g of suspension with a solids concentration of 1.50 g kg⁻¹. Various amounts of 0.01 M KH₂PO₄ solution were added to pH-6 suspensions while vigorously stirring. A pH of 6.0 ± 0.05 was maintained by periodically adding 0.01 M HCl or KOH (Khare et al., 2004) while equilibrating suspensions by shaking on a reciprocating water-bath shaker at 2 s⁻¹ for 42 h. After centrifuging at 16,300 g for 15 min, supernatant solutions were filtered through 0.2 μm Millipore polycarbonate filter membranes. Dissolved PO₄ was analyzed using the molybdate colorimetric (Murphy-Riley) procedure (Olsen and Sommers, 1982), and sorbed PO₄ was determined as loss from solution.

Phosphorus K-Edge XANES Data Collection and Analysis

Samples for P K-edge XANES analysis were prepared at concentrations between 42 to 162 mmol P mol⁻¹ Al+Fe using identical procedures of isotherm experiments, but with a sample mass of 200.00±0.05 g. A portion (~0.2 g) of the moist solids was collected after centrifugation, mounted in acrylic sample holders, covered with 5 μm polypropylene x-ray film to inhibit desiccation, and stored at 4 °C until data collection (Khare et al., 2004). Spectra were collected at Beamline BL16A1 at the National Synchrotron Radiation Research Center in Hsinchu, Taiwan. Details for data collection and processing are given in Appendix.
RESULTS

Characteristics of AFH

X-ray diffraction results for the 0- and 10-AFH samples showed two broad peaks centered at 2.6 and 1.5 Å (Figure 2.1), consistent with two-line ferrihydrite (Schwertmann and Cornell, 2000). These peaks are also discernable for the 20-AFH sample (Figure 2.1), but samples with Al $\geq$ 50 mol% show no evidence for ferrihydrite or crystalline solids. Moreover, no evidence for crystallization of Al-hydroxides was shown by FTIR spectra in any samples (Figure A2.1 in Appendix) (White and Roth, 1986). The disproportional loss of diagnostic ferrihydrite peaks between 10 and 20 mol% Al indicates a decrease of structural ordering in the AFH samples. However, sample dilution with non-crystalline Al-hydroxide at Al proportions of $\geq$ 50 mol% could mask diffraction peaks from ferrihydrite, if present.

Transmission electron microscope images as exemplified in Figure 2.2 show a change in aggregate structure of AFH with increasing proportion of Al. In particular, a distinct transition from larger, more compact aggregates to smaller, more diffuse aggregates is apparent between the 50-AFH and 75 AFH samples. The TEM images show morphological consistency between the 20- and 50-AFH samples and ferrihydrite (0-AFH), and between the 75-AFH sample and non-crystalline Al-hydroxide (100-AFH). However, there was no clear trend in BET surface areas measured on the same freeze-dried samples: 187±11, 181±8, 227±15, 184±8, and 146±8 m$^2$ g$^{-1}$ for the 0-, 20, 50-, 75- and 100-AFH samples, respectively. For reference, Anderson and Benjamin (1990) measured BET surface areas of 186-201, 297, and 41 m$^2$ g$^{-1}$ for Al/Fe-hydroxide co-precipitates containing 0, 50, and 100
mol% Al. However, Wang et al. (2003) showed that dehydration of poorly crystalline Al-oxyhydroxide samples caused an underestimate in BET surface areas accessible to water in hydrated samples.

The near-surface proportions of Al relative to Fe [Al/(Al+Fe)] detected by XPS analysis were 0, 45, 72, 83, and 100 mol% for the 0-, 20-, 50-, 75-, and 100-AFH samples. These results indicate a 2.3-, 1.4-, and 1.1-fold enrichment of surface Al relative to total Al in the 20-, 50-, and 75-AFH co-precipitates, respectively. Enrichment of Al on particle surfaces was also reported by other co-precipitates with equal Al/Fe molar ratios (Anderson and Benjamin, 1990; Violante et al., 2009). The greater consistency of near-surface Al and Fe with increasing proportion of Al in co-precipitates could result from disaggregation of particles (Figure 2.2), increase in Al-hydroxide domain size, or decrease in Fe-hydroxide domain size.

**Iron K-Edge EXAFS Data of AFH**

As a complement to XRD, Fe K-edge EXAFS data reveal the local bonding environments of Fe in AFH samples. The $k^3$-weighted EXAFS spectra show a systematic decrease in the amplitudes of oscillations at 5.0 and 7.5 Å$^{-1}$ with increasing Al proportion (Figure 2.3a - denoted by arrows); the latter implies a decreasing number of corner-sharing FeO$_6$ linkages (Toner et al., 2009). The high-shell peak with one shoulder in Fourier transformed magnitude portion (Figure 2.3b) is consistent with that of two line-ferrihydrite (Manceau and Drits, 1993; Toner et al., 2009). All EXAFS spectra were fit using one Fe-O path in the first shell, and two Fe-Fe and three Fe-O paths in the higher shells (Table 2.1).
The agreements between EXAFS spectra and fitting results are shown in Appendix Figure A2.2, wherein 0-AFH was taken as an example to illustrate the contribution of each path to the EXAFS fitting (Figure A2.3 in Appendix).

Coordination numbers for the first-shell Fe-O paths were averaged as $5.7 \pm 0.3$ at an average distance of $1.98 \, \text{Å}$ for all AFH (Table 2.1). With increasing Al proportion, the fitted interatomic distances of Fe-Fe shells at 3.05 and 3.43 Å, corresponding to edge- and corner-sharing (ES and CS) FeO$_6$ linkages (Manceau and Combes, 1988; Manceau and Drits, 1993), remained essentially constant (Table 2.1); however, the CN for ES FeO$_6$ decreased from $2.8 \pm 0.4$ to $1.9 \pm 0.4$ (Table 2.1). Although CN can generally not be determined to greater than 10% accuracy in EXAFS analysis (Kelly et al., 2008), and CN for 3.43-Å Fe-Fe paths (CS FeO$_6$) were not significantly different within uncertainties, the decreasing trend in the modeled CN with increasing Al proportion indicates that Al co-precipitation influenced the local coordination of Fe in AFH. Besides the reduction in CN, a decrease in EXAFS amplitude (Figure 2.3b) could also be ascribed to the increased structural disorder, modeled through the $\sigma^2$ parameters, and/or to the isomorphic substitution of Al, a weaker backscatterer, for Fe. The $\sigma^2$ parameters for Fe-Fe paths were the same within uncertainties across all samples (data not shown) and were therefore combined into a single model parameter fitted as $0.011 \pm 0.002 \, \text{Å}^2$ (Table 2.1). Moreover, substituting Al into either or both Fe-Fe paths gave no improvement in the EXAFS fits (Appendix). In fact, Fe-Al distances are shorter than Fe-Fe distances due to the smaller ionic radius of octahedrally coordinated Al(III) (0.53Å) compared with Fe(III) (0.73 Å) (Shannon and Prewitt, 1969), causing a ~0.03 Å decrease in unit-cell dimensions while Al substitution increases to 20 mol%, as has been observed in
crystalline Fe-oxides (Schulze, 1984). The difference in our fitted Fe-O and Fe-Fe distances were within 0.006 Å across all samples (Table 2.1), suggesting that Al substitution for structural Fe was negligible. Apparently, rapid synthesis of AFH at ambient temperature did not favor isomorphic substitution of Al for Fe as has been found for crystalline Fe-hydroxides synthesis under hydrothermal conditions (Schwertmann and Cornell, 2000).

A shift in Fe-Fe CN with increasing Al can alternatively be attributed to a change in Fe-hydroxide domain size (Appendix). Because of the nano-scale domain size (~2 nm) of two-line ferrihydrite (Michel et al., 2007), a significant proportion of Fe occurs as terminal FeO₆ at domain surfaces. For such small domains, a change in domain size can be detected by EXAFS because terminal FeO₆ has fewer Fe neighbors (Corrias et al., 2000; Greegor and Lytle, 1980). The decreasing CN for Fe-Fe coordination (Table 2.1) indicates a trend of decreasing domain size with the presence of Al, suggesting that Al disrupts polymerization of FeO₆ octahedra, which agrees with the results implied by XRD data (Figure 2.1). A similar trend was found for the 7-d-aged AFH, wherein greater Al addition led to a greater proportion of Fe present in finer solids (< 10 nm) (Colombo and Violante, 1996). This effect is understandable if Fe(OH)₃ precipitation precedes that of Al(OH)₃ during co-precipitation. Aqueous speciation modeling of mixed Al/Fe solutions using Visual Minteq (Felmy et al., 1984; Gustafsson, 2006) shows that saturation indices of ferrihydrite were more than five orders-of-magnitude greater than that of non-crystalline Al-hydroxide (data no shown), consistent with solubility product constants [Fe(OH)₃ = 10⁻³⁹; Al(OH)₃ = 10⁻³⁴ - (Lindsay, 1979)]. In the absence of kinetic limitations, these calculations suggest that ferrihydrite would precipitate first (Rengasamy and Oades, 1979). Accepting this threshold, AFH might
comprise Fe-rich cores with Al-rich surfaces, consistent with the Al-dominant composition on near-surfaces of AFH determined using XPS analysis. Note that Al acted more like a discrete phase rather than a monolayer with Fe-hydroxides, because Al shows no evidence to cover entire surfaces of AFH samples, even in the co-precipitate containing 75 mol% Al.

**Phosphate Sorption Isotherms**

Figure 2.4 shows PO₄ sorption isotherms on AFH samples along Freundlich isotherm models (Sposito, 1984) that were either fit to the data or predicted from linear combinations of models for the pure end members (0- and 100-AFH) (Khare et al., 2004). The maximum of sorption observed increased with increasing Al proportion, from 229 to 338 mmol mol⁻¹ Al+Fe (2490 to 4050 mmol kg⁻¹) for 0- and 100-AFH, respectively. Isotherms of AFH were nearly identical for Al proportions up to 50 mol%, then increased significantly when Al became dominant (75 mol%). As described by Khare et al. (2005), a weighted linear combination of Freundlich models from end members should be consistent with the isotherms for mineral mixtures if the two sorbents behaved independently with regard to PO₄ sorption. For our AFH, the predicted PO₄ sorption is 1.1- to 1.3-fold greater than observed isotherms (Figure 2.4), with the greatest discrepancy shown by the sample of 50-AFH. The discrepancy between the data and the linear-combination models agrees with interacting (associated) rather than independent Al- and Fe-hydroxide domains.
Phosphorus K-Edge XANES Data of Sorbed PO₄

Normalized P K-edge XANES spectra for PO₄ sorbed on AFH samples were characterized by a sharp increase in absorption near 2151 eV terminating as a strong white-line peak at 2152 eV, wherein no essential change in white-line intensity was observed with increasing PO₄ concentration (Figure A2.6 in Appendix).

The pre-white line region of the P K-edge XANES spectra has been used to quantify the relative proportion of PO₄ bonded with Al(III) vs. Fe(III) using linear combination fitting (LCF) (Khare et al., 2005; Khare et al., 2004). Iron(III)-bonded PO₄ gives a unique feature at 2144-2149 eV (Figure 2.5), which becomes more intense with increasing number of Fe(III)-PO₄ bonds (Khare et al., 2007). For a given co-precipitate, the intensity of the pre-white line feature is intermediate between those for PO₄ sorbed on 0- and 100-AFH and substantially independent of sorbed PO₄ concentration (Figure 2.5a-c). For a given PO₄ concentration, the intensity of this pre-white line feature decreased systematically with increasing Al proportion in co-precipitates (Figure 2.5d), indicating a trend of increasing PO₄ bonding to Al(III) relative to Fe(III).

Results of LCF show that, with increased sorbed PO₄ concentration, the relative proportions of Al(III)- and Fe(III)-bonded PO₄ remained essentially constant for these three AFH samples, as indicated by linear regression fit with $r^2 \geq 0.98$ (Figure 2.6, Table A2.2 in Appendix). Due to the interactive polymerizations of Al- and Fe-hydroxides implied from structural characterization of AFH samples, using Al/Fe molar ratios in AFH samples to gauge preferential PO₄ bonding seems more appropriate for co-precipitation systems than using maximum sorption capacity of end member minerals, as was done for physical
mixtures (Khare et al., 2005; Khare et al., 2004). If PO₄ had equal access and no bonding preference for Al and Fe in the systems, PO₄ associated with Al vs. Fe is expected to be in proportion to the metal amount in the co-precipitates. For example, the 20-AFH sample would have 20% Al-associated PO₄ and 80% Fe-associated PO₄. However, 37-48% of Al-bonded PO₄ was found on 20-AFH (Figure 2.6, Table A2.2 in Appendix), indicating an approximately two-fold preferential bonding of PO₄ to Al. A similar but weaker Al-preferential PO₄ bonding was shown for the 50-AFH samples (~ 1.2-fold – Figure 2.6, Table A2.2 in Appendix). For the 75-AFH samples, however, PO₄ showed a non-preferential bonding to either Al or Fe (Figure 2.6).

**DISCUSSION**

**Phosphate Bonding on AFH**

Molecular configuration of sorbed PO₄ was determined using P K-edge XANES data. The white line in XANES spectra has been attributed to an electronic transition from a P 1s atomic orbital into P(3p)-O(2P) or P(3p)-O(2P)-Al(3P) antibonding molecular orbitals for Al(III)- or Fe(III)-bonded PO₄ (Khare et al., 2007). In both systems, the white-line intensity is lower and the full width at half-maximum height (fwhm) is greater for PO₄ precipitates than adsorbed PO₄ (Khare et al., 2005). For our sorbed PO₄, its white-line intensity stayed essentially consistent, irrespective of sorbed PO₄ concentrations (Figure A2.6 in Appendix). Moreover, our data show a greater white-line intensity and a narrower fwhm (~1.8 eV) compared with that of precipitated PO₄ on non-crystalline Al-hydroxide (fwhm = 2.4 eV).
(Khare et al., 2005). These results suggest that PO$_4$, with concentration $\leq 170$ mmol mol$^{-1}$ Al+Fe (1500 mmol kg$^{-1}$), was mainly retained by adsorption rather than precipitation on AFH. Aluminum-PO$_4$ precipitation from PO$_4$ reacted with non-crystalline Al-hydroxide was found in other studies (Khare et al., 2005; Lookman et al., 1994) but not in our P K-edge XANES samples. That is presumably because our freshly precipitated Al-hydroxide had a greater PO$_4$ sorption capacity than that of aged Al-hydroxide used in other studies (Khare et al., 2005; Lookman et al., 1994). Additionally, the maximum PO$_4$ addition in our XANES samples was 46% of sorption capacity, which is less than the addition of 53% and 200% of capacity used by Khare et al. (2005) and Lookman et al. (1994).

Beyond the adsorption mechanism that was responsible for the PO$_4$ retention on our AFH samples, PO$_4$ bonding could be further distinguished as Al- or Fe-bonded species. Results of LCF show that PO$_4$ tended to bond with Al in 20-AFH, but such trend decreased with increasing Al mol%, resulting in a non-preferential PO$_4$ bonding to either Al or Fe in 75-AFH (Figure 2.6). As indicated by the nearly 1:1 relationship (slope = 0.95, $r^2 = 0.98$) between the proportion of Al-bonded PO$_4$ and the near-surface composition of Al suggested from XPS analysis (Figure 2.7), PO$_4$ bonding seemed to be mainly controlled by the accessibility of elements on co-precipitates. Accordingly, while Al increased from 20 to 75 mol%, the decreasing trend for PO$_4$ bonding to Al could be accounted for the decreasing surface-accessibility of unit Al as discussed earlier. The downward deviation for our linear regression fit from the 1:1 relationship (Figure 2.7) implied that, based on the element accessibility, other factors may partially affect PO$_4$ distribution. For example, the decreasing structural ordering and decreasing domain size of Fe-hydroxides disrupted by the surface-
associated Al may enhance the availability of Fe for PO₄ bonding. Thus, the most likely explanation for the lack of PO₄ bonding preference to Al or Fe in the 75-AFH sample lies in the combination of the relatively lower accessibility of unit Al and the relatively higher availability of unit Fe. Moreover, such non-preferential PO₄ distribution implied that Al and Fe domains act more independently for PO₄ sorption, although Fe still inhibited PO₄ sorption on Al-hydroxide as indicated by the deviation between measured and predicted sorption isotherms for the 75-AFH sample (Figure 2.4). Beyond PO₄ sorption properties of pure metal-hydroxides, our collective results demonstrated a unique PO₄ sorption behavior that was induced along the structural interactions between Al- and Fe-hydroxides in co-precipitation systems.

CONCLUSIONS

This study showed that association of Fe(III) and Al(III) in non-crystalline co-precipitates, as expected in nature, significantly changes the PO₄ adsorption properties relative to pure, isolated analogs. Our results indicate that differential precipitation of Fe- vs. Al-hydroxide domains affected both sorption capacities and preferential binding of PO₄ by Fe(III) vs. Al(III), depending on the proportion of each metal present. These interactions could be potentially important in regulating dissolution of PO₄ under reducing conditions, where Fe(III)-PO₄ bonding is expected to be dissociated by Fe(III) reduction to Fe(II). Murray and Hesterberg (2006) showed that Al(III) inhibited Fe(III) reduction and PO₄
dissolution, and our study further illustrated that interactions between Al and Fe in co-precipitated metal systems strongly influence PO₄ adsorption.
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Table 2.1. Structural parameters obtained from Fe K-edge EXAFS fitting analysis for Al/Fe-hydroxide co-precipitates with 0, 20, 50, and 75 mol% of total metal as Al (0-, 20-, 50-, 75-AFH).\(^a\)

<table>
<thead>
<tr>
<th>Al mol%</th>
<th>Path</th>
<th>R (Å)(^b)</th>
<th>CN(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (0-AFH)</td>
<td>Fe-Fe1</td>
<td>3.05 (0.006)</td>
<td>2.8 (0.4)</td>
</tr>
<tr>
<td></td>
<td>Fe-Fe2</td>
<td>3.43 (0.006)</td>
<td>1.4 (0.6)</td>
</tr>
<tr>
<td>20 (20-AFH)</td>
<td>Fe-Fe1</td>
<td>3.05 (0.006)</td>
<td>2.4 (0.4)</td>
</tr>
<tr>
<td></td>
<td>Fe-Fe2</td>
<td>3.43 (0.006)</td>
<td>1.7 (0.5)</td>
</tr>
<tr>
<td>50 (50-AFH)</td>
<td>Fe-Fe1</td>
<td>3.05 (0.006)</td>
<td>2.0 (0.3)</td>
</tr>
<tr>
<td></td>
<td>Fe-Fe2</td>
<td>3.43 (0.006)</td>
<td>1.4 (0.5)</td>
</tr>
<tr>
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<td>1.9 (0.4)</td>
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<tr>
<td></td>
<td>Fe-Fe2</td>
<td>3.43 (0.006)</td>
<td>1.2 (0.6)</td>
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</tbody>
</table>

\(^a\) The amplitude reduction factor (S\(_0\)^2), transferred from first-shell fitting of hematite, was fixed at 0.83 ± 0.18 for all EXAFS fitting. All samples were fit using one energy shift parameter (\(\Delta E = -2.31 \pm 0.38\) eV). Besides two Fe-Fe paths, all spectra were fit using one Fe-O (Fe-O1) and three Fe-O (Fe-O2, -O3, and -O4) paths for the first- and higher-shell peaks. One floated mean-square displacement of interatomic distance (\(\sigma^2\)) was designated for each group of paths and shared across all samples, which is 0.011 (0.001), 0.011 (0.002), and 0.017 (0.003) Å\(^2\) for the first-shell Fe-O and higher-shell Fe-Fe and Fe-O paths. The normalized sum of the squared residuals of the overall fit (\(R = \sum (\text{data-fit})^2 / \sum \text{data}^2\)) was 1.8% for all fits.

\(^b\) Interatomic distance. Path distances of Fe-O1, -O2, -O3, and -O4 were fit as 1.98 (0.004), 3.41 (0.006), 3.62 (0.006), and 3.80 (0.006) Å. The parameters of \(\Delta R\) for the first- and the higher-shell paths were fit to a separate common value across all samples.

\(^c\) Degeneracy (coordination number, CN). CN for Fe-O1 was 5.7 (0.3), which was fit using a common value across all samples. CNs for Fe-O2, -O3, and -O4 were constrained along with that of edge-sharing (Fe-Fe1) and corner-sharing (Fe-Fe2) octahedra. CNO2 = 1/4*CNFe2; CNO3 = 1/4*CNFe2 + 1/2*CNFe1; CNO4 = 1/2*CNFe2 + CNFe1 (Appendix).
Figure 2.1. X-ray diffraction patterns for Al/Fe-hydroxide co-precipitates with 0, 10, 20, 50, 75, and 100 mol% of total metal as Al (0-, 10-, 20-, 50-, 75-, 100-AFH). Data were collected using unresolved Cu Kα radiation.
Figure 2.2. TEM images of Al/Fe-hydroxide co-precipitates with 0, 20, 50, 75, and 100 mol% of total metal as Al (0-, 20-, 50-, 75-, 100-AFH).
Figure 2.3. The (a) Fe K-edge EXAFS spectra and (b) Fourier transformed magnitude of Al/Fe-hydroxide co-precipitates with 0, 20, 50, and 75 mol% of total metal as Al (0-, 20-, 50-, 75-AFH).
Figure 2.4. Phosphate sorption isotherms at pH 6.0 ± 0.05 and corresponding Freundlich models (solid lines) for Al/Fe-hydroxide co-precipitates with 0, 20, 50, 75, and 100 mol% of total metal as Al (0-, 20-, 50-, 75-, 100-AFH). The A and β parameters for the Freundlich models are (q_i = A c_i^β, where q_i is the fitted sorbed PO_4 concentration for a given dissolved concentration c_i): 84 and 0.12 for 0-AFH; 91 and 0.12 for 20-AFH; 87 and 0.12 for 50-AFH; 132 and 0.10 for 75-AFH; 170 and 0.10 for 100-AFH. Dash lines represent predicted PO_4 sorption isotherms derived from weighted combinations of Freundlich models for end members of ferrihydrite (0-AFH, q_0) and Al-hydroxide (100-AFH, q_{100}) based on the relative proportions of Al (x) and Fe (y) in co-precipitates (Khare et al., 2004), i.e., q_{x,predicted} = y q_0 + x q_{100}. 
Figure 2.5. Pre-white line region of normalized XANES spectra for PO₄ sorbed at various concentrations (in mmol P mol⁻¹ Al+Fe shown as numbers in the legend) on Al/Fe-hydroxide co-precipitates with Al contents of (a) 20 (20-AFH), (b) 50 (50-AFH), and (c) 75 (75-AFH) mol% of total metal concentration; and for (d) PO₄ sorbed at ~160 mmol P mol⁻¹ Al+Fe across all samples (162, 159, and 158 mmol P mol⁻¹ Al+Fe for 20-, 50-, and 75-AFH). Spectra were compared with end members of PO₄ sorbed on ferrihydrite (0-AFH) and on non-crystalline Al-hydroxide (100-AFH) at concentrations of 170 mmol P mol⁻¹ Fe and 154 mmol P mol⁻¹ Al.
Figure 2.6. Trends in PO₄ associated with Fe(III) vs. Al(III) on Al/Fe-hydroxide co-precipitates with 20, 50, and 75 mol% of total metal as Al (20-, 50-, 75-AFH) determined by the linear combination fitting across the pre-while line region as a function of total sorbed PO₄. Linear regression fits to the data are shown as solid lines, and dashed lines are reference lines (RL) representing the expected Fe(III)-associated PO₄ if PO₄ bonds with Fe(III) and Al(III) in proportion to their concentrations in the co-precipitates. For the 75-AFH sample, the regression and reference lines coincide.
Figure 2.7. The trend of PO$_4$ associated with Al(III) represented by the proportion of total sorbed PO$_4$ on Al/Fe-hydroxide co-precipitates with 0, 20, 50, 75, and 100 mol% of total metal as Al (0-, 20-, 50-, 75-, 100-AFH) as the function of near-surface composition of Al in each AFH determined using the x-ray photoelectron spectroscopy. The proportion of Al-bound PO$_4$ on 20-, 50, and 75-AFH was averaged from that with various sorbed PO$_4$ concentrations reported in Table A2.2 in Appendix.
APPENDICES
Chemical and Mineralogical Analyses for Al/Fe-Hydroxide Co-Precipitates

Freeze-dried subsamples of AFH were dissolved in concentrated HCl. The concentrations of Al and Fe determined using flame atomic absorption spectrometry (Schulze, 1984) show that Al/(Al+Fe) molar ratio in each AFH was consistent with that of added salts. For x-ray diffraction (XRD) analysis, subsamples were mounted in 1-mm-thick glass sample holders, and diffraction patterns were recorded using unresolved Cu-Kα radiation with a Rigaku Miniflex diffractometer. Note that the 10-AFH sample was only characterized with chemical analysis and XRD. Near-surface compositions of AFH were measured using x-ray photoelectron spectroscopy (XPS) with a RIBER LAS-3000 spectroscope under monochromatic Al Kα radiation. The energy step size for high resolution scans was 0.1 eV. Surface concentrations of elements (atom percent) were obtained from spectral deconvolution and fitting of O 1s, C1s, Fe 2p3/2, and Al 2p signals from these respective elements. Microscale morphologies of alcohol-dispersed AFH suspensions were analyzed using transmission electron microscopy (TEM - Philips TECNAI 20). Fourier-transform infrared spectra (FTIR) were collected using a Nicolet Nexus 470 spectrometer. Pellets for FTIR analysis were prepared with the oven-dried (110 °C) co-precipitates and KBr powder at a concentration of 10 mg g⁻¹. Single-point Brunauer-Emmett-Teller (BET) surface areas were measured on triplicate samples by N₂ adsorption with a Quantachrome Monosorb analyzer (Carter et al., 1986).
X-ray Absorption Spectroscopy (XAS) Data Collection and Analysis

Iron K-Edge Spectra

Data Collection

The electron storage ring was operating at a maximum current of 300 mA. The synchrotron radiation adjusted with a channel-cut Si(111) monochromator was detuned by > 30% at 200 eV above the Fe K-edge, calibrated to 7112 eV using an Fe foil, and monitored for energy shifts during data collection with the Fe foil as a reference (Kelly et al., 2008). Samples were analyzed in transmission mode using N$_2$(g)-filled ion chamber detectors. Spectra were acquired at photon energies from -200 eV to a wavenumber (k) of 14 Å$^{-1}$ relative to 7112 eV, using a step size of 0.5 eV in the near edge region (-30 to +50 eV) and a step size of k = 0.05 Å$^{-1}$ at higher energies (Kelly et al., 2008).

Data Analysis and EXAFS Fitting

Multiple XAS scans on each sample were aligned, merged, and processed using the Athena program, an interface of IFEFFIT (version 1.2.10) (Newville, 2001; Ravel and Newville, 2005; Sayers and Bunker, 1988). Spectra were baseline corrected using a linear pre-edge function between -200 and -30 eV and a linear or quadratic normalization function between 150 and 630 eV (Kelly et al., 2008). Background were removed from EXAFS spectra using a cubic function with nodes defined by the AUTOBKG function in IFEFFIT (Newville, 2001). The background function was fit to a model for the first-shell Fe-O coordination of the 0-AFH as discussed by Kelly et al. (2008). Fourier transformations of k$^3$-
weighted spectra \([k^2 \chi(k)]\) were taken across a k range of 2.5 to 11.5 Å\(^{-1}\) using a Kaiser-Bessel window with 1 Å\(^{-1}\) sill width. The real and magnitude parts of the Fourier transformed spectra are shown without phase shift correction.

For EXAFS fitting, theoretical scattering paths were obtained via a FEFF calculation (Zabinsky et al., 1995) from the Fe1 site in the structural model of two-line ferrihydrite [2-nm domain - (Michel et al., 2007)]. Our fitting model represented all single scattering Fe-Fe and Fe-O paths having an amplitude \(>15\%\) (relative to the shortest path) to a maximum radial distance of 3.8 Å. To decrease the number of paths and fitting parameters in the model while including essentially structural information from the published ferrihydrite structure, common paths (Fe-Fe or Fe-O) within \(~0.1\) Å were grouped together, and Fe-Fe paths at 3.02 (Fe-Fe1) and 3.40 (Fe-Fe2) Å and Fe-O paths at 1.98 (Fe-O1), 3.38 (Fe-O2), 3.59 (Fe-O3), and 3.77 (Fe-O4) Å were used as structural representatives. Paths of Fe-Fe1 and Fe-Fe2 represent the interatomic distances of edge- and corner-sharing (ES and CS) FeO₆ linkages in the two-line ferrihydrite structure (Manceau and Drits, 1993; Toner et al., 2009). Because the crystallographic structure of two-line ferrihydrite reported by Michel et al. (2007) contains 20% tetrahedral Fe linkages, and ferrihydrite is a precursor of goethite, we used goethite structure as a template to model the correlation in degeneracies between higher-shell Fe-O paths and ES and CS FeO₆. In a goethite structure, a Fe atom is surrounded by two 3.0-Å ES FeO₆ and four 3.4-Å CS FeO₆. These two ES FeO₆ are associated with one \(~3.5\)-Å and two \(~3.8\)-Å Fe-O paths. For four CS FeO₆, Fe-O distances at \(~3.2\), \(~3.5\), and \(~3.8\) Å have degeneracies of one, one, and two. Because the coordination numbers (CN) for higher-shell Fe-O paths vary proportionally with that of ES and CS FeO₆, they could be constrained by
the following equations: CNO2 = 1/4*CNFe2; CNO3 = 1/4*CNFe2 + 1/2*CNFe1; CNO4 = 1/2*CNFe2 + CNFe1. All EXAFS spectra were fit for distances, CN, and mean-square displacement of interatomic distance (σ²) across the R range from 1.0 to 3.5 Å using the Artemis interface (Ravel and Newville, 2005) with a fixed amplitude reduction factor (S₀²) derived from a first shell Fe-O fit of hematite. For those fitted structural parameters that were the same within uncertainties across all samples (data not shown), a common model parameter was used instead to decrease the number of fitted variables (see the footnote in Table 2.1) (Kelly et al., 2008).

**Phosphorus K-Edge Spectra**

**Data Collection**

The storage ring was operated at a fixed current of 250 mA. The synchrotron radiation that passes through a set of focusing mirrors was detuned by 10% at 200 eV above the P(V) K-edge. The channel-cut Si(111) monochromator energy was calibrated to 2222.3 eV based on the first inflection point in the L₃-edge derivative spectra from a Zr foil. Spectra were collected in fluorescence mode between -50 to +300 eV relative to a P(V) K-edge energy at 2151 eV using a step size of 0.1 eV across the absorption edge region (-5 to +10 eV). A He(₉) -purged flight path and a He(₉)-filled Lytle detector were used. Any self-absorption effects were considered to be insignificant because no essential decrease in the white-line peak intensity was observed with increasing sorbed PO₄ concentration (Khare et al., 2004).
Data Analysis and Linear Combination Fitting (LCF)

Multiple XANES scans on each sample were merged and normalized using procedures analogous to that for Fe XAS analysis. The distribution of PO₄ bonding between Al and Fe in AFH samples was determined by LCF with Athena interface (Ravel and Newville, 2005) across the pre-edge region from -7 to -2 eV (Khare et al., 2004). Samples of PO₄ sorbed on ferrihydrite (0-AFH) and Al-hydroxide (100-AFH) at 170 mmol P mol⁻¹ Fe and 154 mmol P mol⁻¹ Al were used as end members in LCF.
Figure A2.1. Fourier-transform infrared spectra for Al/Fe-hydroxide co-precipitates with 0, 20, 50, 75, and 100 mol% of total metal as Al (0-, 20-, 50-, 75-, 100-AFH). Peaks at ~3500, 2420, and 1630 cm$^{-1}$ are O-H stretching, bonded O-H stretching, and H-O-H bending modes; the one at 1380 cm$^{-1}$ is due to C-O stretching of adsorbed CO$_2$ (White and Roth, 1986), which may occur during sample exposure to air. No evidence of crystallization was observed in any AFH samples (White and Roth, 1986).
Figure A2.2. Model fits (solid lines) to Fourier transform of EXAFS spectra (open circles) for Al/Fe-hydroxide co-precipitates with 0, 20, 50, and 75 mol% of total metal as Al (0-, 20-, 50-, 75-AFH). Spectra show similar but weaker oscillations in peaks at 2.5 and 3.0 Å with increasing Al mol%.
Figure A2.3. Model fit (solid lines) to Fourier transform of EXAFS spectra (open circles) for two-line ferrihydrite (0-AFH – Figure 2.1) in the R range of 1.0 to 3.5 Å. Scattering paths fitted using a structural model for two line-ferrihydrite [2-nm domain - (Michel et al., 2007)] illustrate the contribution from absorber atoms (Fe) to surrounding atoms (O and Fe).
Evaluation for Al Isomorphic Substitution in Fe-Hydroxide Structures via EXAFS Analysis

Besides Fe-O paths, the structure model adopted in the EXAFS fitting for Al/Fe-hydroxide co-precipitates consisted of 3.02 Å and 3.40 Å Fe-Fe paths. The strategies examining the presence of Al substitution were to substitute Fe-Fe paths by Fe-Al paths (Table A2.1). The Fe-Al scattering paths calculated from FEFF program are shown in Figure A2.4. Two types of Fe-Al paths were generated: one had same interatomic distance and degeneracy as the Fe-Fe path; the other had same distance but half degeneracy as the Fe-Fe path. Although same degeneracy was given to both Fe-Fe and Fe-Al paths, the magnitude of Fe-Al path was almost half of that of Fe-Fe path due to the relatively lighter atomic number. The other noteworthy feature between Fe-Fe and Fe-Al path was the phase shift in spite of same interatomic distance was assigned to them (Figure A2.4).

Table A2.1. The combination of Fe-Fe and Fe-Al paths used in EXAFS fitting to examine the Al-substitution in Fe-hydroxide for the sample of Al/Fe-hydroxide co-precipitates with 20 mol% of total metal as Al (20-AFH).

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Path at 3.02 Å</th>
<th>Degeneracya</th>
<th>Path at 3.40 Å</th>
<th>Degeneracya</th>
<th>R (%)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Fe-Fe</td>
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<td>Fe-Fe</td>
<td>2</td>
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</tr>
<tr>
<td>1</td>
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<td>2</td>
<td>Fe-Fe</td>
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</tr>
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<td>2</td>
<td>Fe-Al</td>
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<td>Fe-Al</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Fe-Fe</td>
<td>2</td>
<td>Fe-Fe</td>
<td>1</td>
<td>2.2</td>
</tr>
<tr>
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<td></td>
<td></td>
<td>Fe-Al</td>
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</tr>
<tr>
<td>6</td>
<td>Fe-Fe</td>
<td>1</td>
<td>Fe-Fe</td>
<td>1</td>
<td>3.2</td>
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<tr>
<td></td>
<td>Fe-Al</td>
<td>1</td>
<td>Fe-Al</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

*a Default degeneracy used for FEFF calculation was derived from the structure of two-line ferrihydrite (Michel et al., 2007). During the EXAFS fitting, the coordination number of each path was floated.

*b Normalized sum of the squared residuals of the overall fit for 20-AFH ($R = \sum(data-fit)^2/\sum data^2$).
Among these scenarios, the model only including Fe-Fe paths (scenario 0) gave the fitting results for Al/Fe-hydroxide co-precipitates containing 20 mol% Al (20-AFH) with $R$ factor of 1.8 % (Figure A2.5a; see Table 2.1 for structural parameters). With respect to Al contribution, scenarios with Fe-Al paths were not suitable for the EXAFS fitting in that unreasonable structure parameters was obtained. For example, albeit scenario one seemed to be the best model to fit 20-AFH structure (Figure A2.5b), the coordination number of Fe-Al path at 3.02 Å was predicted as $-2.9 \pm 0.8$. Because it is feasible to determine Fe-Al scattering path via fitting Fe K-edge EXAFS spectra of Al-substituted goethite (data not shown), the lack of Fe-Al contribution in co-precipitated Al/Fe-hydroxide was thereby due to its intrinsic structure characteristics rather than the technique limitation. Moreover, it has been well-known that structure shrinkage comes with the Al isomorphic substitution into Fe-(hydr)oxides (Fey and Dixon, 1981). Therefore, a relatively shorter interatomic distance should be found if Al-substitution occurs in structures of Al/Fe-hydroxide co-precipitates, which is, apparently, not the case for our consistent Fe-Fe distances. Conclusively, a significant amount of Al substitution (i.e. isomorphic substitution) into ferrihydrite structure might be ruled out for the co-precipitation systems between Al- and Fe-hydroxides.
Figure A2.4. The Fourier transformed magnitude portion of scattering paths at 3.02 Å for atom pairs of Fe-Fe and Fe-Al with two degeneracy and Fe-Al with one degeneracy.
Figure A2.5. Model fits (solid lines) to Fourier transform of EXAFS spectra (open circles) for Al/Fe-hydroxide co-precipitates with 20 mol% of total metal as Al (20-AFH). Scattering paths fitted using a structural model for two line-ferrihydrite [2-nm domain - (Michel et al., 2007)] illustrate the contribution from absorber atoms (Fe) to surrounding atoms (O / Fe / Al). (a) 20-AFH was fitted with the model only including Fe-O and Fe-Fe paths (scenario 0). (b) 20-AFH was fitted with Fe-O, Fe-Fe, and Fe-Al paths (scenario 1).
Conceptualization of Domain Size for Fe-Hydroxides Based on the Coordination Number of Fe Octahedral Linkages Obtained from EXAFS Analysis

Based on the approach used in previous studies (Rose et al., 2002; Thoral et al., 2005), the domain size can be conceptualized according to the coordination number (CN) of FeO$_6$ linkages obtained from the EXAFS analysis. As Al increased from 0 to 75 mol%, the CN of edge-sharing (ES) FeO$_6$ linkages decreased from 2.8±0.4 to 1.9±0.4, but that for corner-sharing (CS) FeO$_6$ linkages showed no trend and remained constant within uncertainties across all co-precipitates (Table 2.1). The decrease in the CN of ES FeO$_6$ implied a decreasing domain size with increasing Al co-precipitation, shown as the sketched models below. Given that CN cannot be determined to greater than 10% accuracy in EXAFS analysis (Kelly et al., 2008), the following models do not represent the exact Fe domain size.

Sample of 0-AFH with ES Fe = 2.8±0.4; CS Fe = 1.4±0.6

Sample of 75-AFH with ES Fe = 1.9±0.4; CS Fe = 1.2±0.6
Figure A2.6. Normalized P K-edge XANES spectra for PO₄ sorbed at various concentrations (in mmol P mol⁻¹ Al+Fe shown as numbers in the legend) on Al/Fe-hydroxide co-precipitates with 0, 20, 50, 75, and 100 mol% of total metal as Al (0-, 20-, 50-, 75-, 100-AFH).
Table A2.2. Phosphate associated with Fe(III) and Al(III) on Al/Fe-hydroxide co-precipitates with 0, 20, 50, 75, and 100 mol% of total metal as Al (20-, 50-, 75-AFH) calculated using linear combination fitting of normalized P K-edge XANES spectra.\(^a\)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sorbed PO(_4) (mmol mol(^{-1}) Al+Fe)</th>
<th>% Associated with Fe</th>
<th>% Associated with Al</th>
<th>(\chi^2)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>58 ± 0.4</td>
<td>42 ± 0.4</td>
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</tr>
<tr>
<td></td>
<td>66</td>
<td>52 ± 0.4</td>
<td>48 ± 0.4</td>
<td>0.0005</td>
</tr>
<tr>
<td></td>
<td>88</td>
<td>60 ± 0.3</td>
<td>40 ± 0.3</td>
<td>0.0004</td>
</tr>
<tr>
<td></td>
<td>131</td>
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</tr>
<tr>
<td></td>
<td>162</td>
<td>63 ± 0.3</td>
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</tr>
<tr>
<td>50-AFH</td>
<td>44</td>
<td>48 ± 0.8</td>
<td>52 ± 0.8</td>
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</tr>
<tr>
<td></td>
<td>65</td>
<td>38 ± 0.4</td>
<td>62 ± 0.4</td>
<td>0.0006</td>
</tr>
<tr>
<td></td>
<td>87</td>
<td>42 ± 0.5</td>
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<tr>
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<td>130</td>
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<tr>
<td></td>
<td>159</td>
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<td>75-AFH</td>
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<tr>
<td></td>
<td>158</td>
<td>26 ± 0.4</td>
<td>74 ± 0.4</td>
<td>0.0006</td>
</tr>
</tbody>
</table>

\(^a\) Standards using for linear combination fitting was ferrihydrite (0-AFH) and non-crystalline Al-hydroxide (100-AFH) with sorbed PO\(_4\) concentration of 170 mmol mol\(^{-1}\) Fe and 154 mmol mol\(^{-1}\) Al reacted at pH 6.0 and 0.01 M KCl background electrolyte. Deviations (±) represent uncertainties calculated by Athena from the linear combination fitting.
CHAPTER 3

Microbial Reduction of Non-Crystalline Al/Fe-Hydroxide Co-Precipitates

ABSTRACT

Biogeochemical cycling of Fe, which essentially influences fates of trace elements and plant nutrients, is often tied with microbial reduction. However, bioreduction mechanisms for Fe-(hydr)oxides co-precipitated with the redox-inactive Al-(hydr)oxides are largely unknown. In this study, we aimed to determine the impact of Al co-precipitation on bioreducibility of Fe-hydroxides in relation to local structural changes of Fe domains. Bioreduction of Al/Fe-hydroxide co-precipitates containing 0 to 100 mol% of Al/(Al+Fe) molar ratios was promoted by *Shewanella putrefaciens* CN32. After a 168-h incubation, total Fe(II) production increased 40-fold as co-precipitated Al increased from 0 to 50 mol%. Influences of co-precipitated Al also emerged in the partitioning of biogenic Fe(II), wherein increasing Al mol% induced smaller proportions of total Fe(II) distributed in solid phases. During bioreduction, a local structural development in Fe domains was found in pure Fe-hydroxides but not in samples containing Al. Here, co-precipitated Al tended to act as a barrier against development of Fe-hydroxide structure, potentially increasing the active surface sites. By optimizing availability of Fe domains, co-precipitated Al enhanced Fe(III) bioreduction.
INTRODUCTION

Poorly-crystalline Al- and Fe-(hydr)oxides are usually characterized by larger surface areas compared with phyllosilicates and crystalline oxide minerals, and thereby are capable of serving as dominant sorbents for nutrients (e.g., P), metals (e.g., Cu, Zn), and metalloid (e.g., As) in soils (Violante and Pigna, 2002). As soils become more reduced, the fate and availability for such sorbates are expected to be controlled by the relative association with redox-active Fe-(hydr)oxides or with redox-inactive Al-(hydr)oxides (Darke and Walbridge, 2000; Holford and Patrick, 1981; Patrick and Khalid, 1974).

The biogeochemical cycling of Fe in aquatic and terrestrial environments is often driven by and coupled with microbial processes. Geochemical evidence indicates that Fe(III) is one of the first terminal electron acceptors of global significance in microbial metabolism (Vargas et al., 1998). Via Fe reduction, bacteria obtain energy to support their growth. Dissimilatory reduction of Fe-(hydr)oxides has been documented for a large number of microorganisms in a wide range of environments (Lovley, 1997), which has a significant effect on geochemistry by affecting the speciation and mobility of metals such as U and Cr (Lovley, 1995; Lovley, 1997). Although these dissimilatory iron-reducing bacteria (DIRB) can utilize both poorly-crystalline and crystalline Fe-(hydr)oxides as electron acceptors (Fredrickson et al., 1998; Lovley and Phillips, 1986; Zachara et al., 1998), particles with short-range structural order such as ferrihydrite are more available to DIRB due to the greater solubility and relatively larger surface areas (Lovley and Phillips, 1986). A positive correlation was found between the bioreducibility and surface areas of Fe-(hydr)oxides
(Roden and Zachara, 1996). In contrast to ferrihydrite, which is often fully reduced or transformed to secondary Fe(II) containing mineral solids, crystalline Fe(III)-oxide minerals such as goethite and hematite are only partially reduced in batch experiments with DIRB (Fredrickson et al., 1998).

The impacts of Al in bioreduction of Fe(III)-(hydr)oxides varied with the application methods. For Al-substituted Fe-(hydr)oxides, Fe(III) bioreducibility was either inhibited or not influenced by the presence of Al (Bousserhine et al., 1999; Kukkadapu et al., 2001; Urrutia et al., 1999). Inhibition generally resulted from the sorption of solubilized Al that acted to passivate surfaces of Fe-(hydr)oxides and/or microbes. However, while Al-(hydr)oxides were placed separately from Fe minerals and microbes in dialysis bags, they acted as Fe(II) sinks to decrease Fe(II) accumulation on surfaces of particles and microbes, and thereby enhanced Fe(III) bioreducibility (Urrutia et al., 1999). Conclusively, Al can potentially influence Fe(III) bioreduction by either delaying electron transfer via attaching to particle/cell surfaces, or by promoting Fe(III) reducibility via retarding the formation of Fe(II) coating on particle/cell surfaces.

As indicated by our previous results (Chapter 2), in the system of non-crystalline Al/Fe-hydroxide co-precipitates, the degree of structural ordering in Fe-hydroxides decreased with increasing Al proportion. It has not been fully resolved whether such structural changes can induce further structural transformation in Fe domains during Fe(III) bioreduction, and thereby alter bioreducibility of Fe(III). X-ray diffraction (XRD) and Mössbauer spectroscopy have been used to examine mineralogical evolution of Fe-(hydr)oxides in most bioreduction studies (Fredrickson et al., 1998; Kukkadapu et al., 2001; O'Loughlin et al., 2007). However,
structural characterization of non-crystalline particles is limited for both techniques. Our objective here was to determine how co-precipitated Al affects the extent of microbial Fe(III) reduction and Fe dissolution in relation to the local structural transformation of Fe-hydroxides. We used Fe K-edge x-ray absorption spectroscopy (XAS), including x-ray absorption near edge structure (XANES) and extended x-ray absorption fine structure (EXAFS) spectroscopy, to examine the molecular-scale structural changes based on the advantage that XAS provides insights of local configuration between central Fe and surrounding atoms.

MATERIALS AND METHODS

Synthesis of Non-Crystalline Al/Fe-Hydroxide Co-Precipitates (AFH)

The AFH samples were synthesized following the revised ferrihydrite synthesis procedure as described in Chapter 2 with the exception that 0.05 instead of 0.01 M KCl was used as a background electrolyte here. Briefly, mixtures of Fe(NO$_3$)$_3$·9H$_2$O and Al(NO$_3$)$_3$·9H$_2$O solutions with total metal concentration of 0.2 mol Al+Fe L$^{-1}$ and Al/(Al+Fe) molar ratios of 0, 0.1, 0.2, 0.5, 0.75, and 1.0 (0-, 10-, 20-, 50-, 75-, and 100-AFH) were hydrolyzed with 1 M KOH to pH 7.5 and vigorously stirred for 0.5 h. Precipitates were washed with 1 M KCl and subsequently with 0.05 M KCl solution. Final suspensions were dispersed in 0.2 L of 0.05 M KCl solution, adjusted to pH 5.5, and stored at 4 ºC for a maximum of 2 weeks. Details in the chemical and mineralogical analyses for AFH samples were given in Chapter 2. The Al/(Al+Fe) molar ratio measured for each precipitate was
consistent with that of added salts, indicating that all Al and Fe were incorporated into solid phases. Fe-hydroxides in 0-AFH samples were characterized as 2-line ferrihydrite using XRD, but the diagnostic intensity of the broad peaks for ferrihydrite disproportionally decreased with increasing Al proportion. In samples with Al ≥ 50 mol%, only featureless patterns indicative of non-crystalline solids were detected (Chapter 2).

**Microorganism and Culture Conditions**

Iron-reducing bacteria *Shewanella putrefaciens*, strain CN32 (CN32), was provided by Dr. Edward O’Loughlin (Biosciences Division, Argonne National Laboratory). Microbes were grown aerobically in flasks containing 150 mL of tryptic soy broth (30 g L⁻¹). Suspensions were incubated on a wrist action shaker at a rate of 2 s⁻¹ and ambient temperature. Cells were harvested by transferring a 16 h-old culture (late log phase) to 250-mL polycarbonate bottles and centrifuging at 15,680 g and 20 °C for 7 min. Cell pellets were re-suspended in 0.05 M KCl and washed three times with 0.05 M KCl by shaking and centrifuging (O’Loughlin et al., 2007). The density of each final cell suspension was determined by absorbance at 420 nm (Royer et al., 2002). All equipment and solutions used were sterile.

**Bioreduction Experiments**

Bioreduction experiments performed in sterile 125-mL amber serum vials sealed with rubber septa and aluminum crimp caps were initiated at pH 5.5. Each vial held 100 mL of suspension containing 50 mmol Al+Fe L⁻¹ as suspended AFH samples, 0.025 M sodium
formate as an electron donor, 0.05 M 2-(N-Morpholino)ethanesulfonic acid potassium salt (MES; pKa = 6.1) as a pH buffer, and 0.05 M KCl as a background electrolyte. Except for AFH suspensions, media components were either autoclaved (KCl) or filter sterilized (formate, MES) using a syringe filtration system equipped with a 0.2 µm Millipore polycarbonate filter membrane. Before inoculation, sealed vials were spiked using a sterile needle and flushed with a stream of filter-sterilized nitrogen [>99.99% N₂(g)] for 15 min to reduce molecular oxygen (Kukkadapu et al., 2001). The experiments were initiated by spiking each vial anoxically with the volume of inoculums needed to achieve a cell density of ~3×10¹¹ cells L⁻¹. Vials were incubated at 25 °C and agitated horizontally on reciprocating water bath shakers at 2 s⁻¹ for 168 h. An aliquot of suspension was collected methodically using sterile syringes at select times. All treatments were prepared in triplicate. Abiotic controls that received 0.05M KCl in place of a CN32 suspension were run simultaneously. All sample handling was done in a glove box under an N₂(g) atmosphere to maintain anoxic conditions.

The suspension pH was determined under an N₂(g) atmosphere using a glass pH electrode. Trends in Fe(III) bioreducibility were monitored by the extent of dissolved and 0.5 M-extractable Fe(II) anaerobically. Dissolved Fe(II) was measured in filtrates that passed through a 0.2 µm Millipore polycarbonate filter membrane. Concentration of 0.5 M HCl-extractable Fe(II), estimated as total Fe(II), was obtained by combining equal volumes of suspension and 1 M HCl, agitating for 24 h in the dark, and centrifuging at 11,270 g for 10 min (Fredrickson et al., 1998). Measurements of Fe(II) in samples were done within 2 h of sampling using the phenanthroline colorimetric method (Olson and Ellis, 1982). To offset the
dilution effect caused by the added HCl, concentration of 0.5 M HCl-extractable Fe(II) was multiplied by factor of two and shown as total Fe(II) in figures.

**Iron K-Edge XAS Data Collection and Analysis**

Bioreduced AFH samples for Fe K-edge XAS analysis were collected after 0.5 and 168 h of incubation and stored under an N$_2$(g) atmosphere at 4 °C to suppress microbial metabolisms (Abboud et al., 2005) until data collection. Details in sample preparation, data collection, and spectra analysis are described previously in Chapter 2. Briefly, Fe K-edge XAS spectra were collected at Beamline X-11B at the National Synchrotron Light Source, Brookhaven National Laboratory, Upton, NY. Spectral background subtraction and normalization were processed with the IFEFFIT package [version 1.2.10, (Newville, 2001)] using the Athena interface (Ravel and Newville, 2005). For EXAFS fitting that was operated using the Artemis interface (Ravel and Newville, 2005), Fe-O and Fe-Fe scattering paths derived from two-line ferrihydrite (Michel et al., 2007) using the FEFF 6 software (Zabinsky et al., 1995) were used as the model structure. The rationale for scattering path selection and development of EXAFS fitting models was discussed in Chapter 2. All of the real and magnitude parts of Fourier transformed spectra are shown without phase shift.
RESULTS

Bioreduction of AFH

Trends in total (0.5 M HCl-extractable) Fe(II) generated during bioreduction of AFH samples are shown in Figure 3.1a. In the absence of Al, the overall reduction extent was 0.6% (0.3 mmol L\(^{-1}\)) of Fe(III) in our freshly prepared 2-line ferrihydrite (0-AFH), which was 18 to 100 times lower than that detected from ferrihydrite bioreduction at various pH and buffer solutions (Table A3.1 in Appendix). Such deviation could be attributed to a lack of nutrients in our incubation medium or to our acidic incubation conditions. Besides KCl, MES (pH buffer), and formate (electron donor), no other amendments such as essential nutrients for microbes (e.g. phosphate) and electron transfer mediators (e.g. organic matter) were added into our incubation media. In addition, the incubation was initiated at pH 5.5 to simulate a biogeochemical process taking place in an acidic condition. However, Fe(III) reductase is less active at pH 5.5 (Gaspard et al., 1998; Grantham et al., 1997) than at pH 6.8-8.0, which is generally used in dissimilatory Fe(III) reduction driven by Shewanella species (Table A3.1 in Appendix). Under the incubation condition initiated at pH 6.8 and amended with defined mineral medium (O'Loughlin et al., 2007) as well as an electron transfer mediator (9,10-anthraquinone-2,6-disulfonate, AQDS), CN32 can reduced 28% of Fe(III) from our fresh-synthesized ferrihydrite (0-AFH) within 15 days (data not shown). For samples containing Al co-precipitation, the total Fe(II) was essentially increased to 12.0 mmol L\(^{-1}\) as Al increased up to 50 mol%. A further increase in Al to 75 mol%, however, decreased total Fe(II) production to a comparable level as the 20-AFH sample (Figure 3.1a).
Under conditions promoting oxidation of formate, bioreduction of Fe-hydroxides is coupled with the consumption of protons.

$$2\text{Fe(OH)}_3(s) + \text{HCOO}^-(aq) + 4\text{H}^+(aq) = 2\text{Fe}^{2+}(aq) + \text{HCO}_3^-(aq) + 5\text{H}_2\text{O}(l)$$

Therefore, total Fe(II) showed a positive correlation \((r^2 = 0.96, p = 0.01)\) (Figure A3.1 in Appendix) with final sample pH that drifted from initial pH 5.5 to pH 6.3 (Figure 3.1a).

During the 168-h bioreduction, aqueous Al across all AFH samples was \(\leq 0.01\) mmol L\(^{-1}\), less than 0.03% of total Al in co-precipitates (data not shown), whereas dissolved Fe(II) was found particularly from those samples containing Al co-precipitation (Figure 3.1b). Aqueous Fe(II) generally increased over time, except in the pure Fe-hydroxide system (0-AFH), wherein dissolved Fe(II) decreased after 12 h of bioreduction (Figure 3.1b inset). As with the total Fe(II) production, dissolved Fe(II) followed an analogous trend with the lowest (0 mmol L\(^{-1}\)) and the highest (10.5 mmol L\(^{-1}\)) concentration produced after a 168-h bioreduction from samples of 0- and 50-AFH (Figure 3.1b). Depending on Al proportion, up to 90% of total Fe(II) was released into solution after 168 h of bioreduction, with the remaining sorbed on residual solids (Figure 3.2). In contrast with the 0-AFH sample, where total Fe(II) resided exclusively on solid phases after 72 h, increasing Al mol\% resulted in \(\leq 40\%\) of total Fe(II) distributed in solid phases after 15 h of bioreduction (Figure 3.2).

The influence of co-precipitated Al on bioreduction was shown by the striking changes in the reducibility of AFH samples and in the partitioning of biogenic Fe(II) to solution and solid phases. Within a 168-h bioreduction period, the 50-AFH sample resulted in the greatest extent of total Fe(III) reduction. However, given that total metal (Al+Fe) concentration was fixed at 50 mmol L\(^{-1}\) across all samples, the proportion of Fe(III) reduced...
increased systematically with increasing proportion of co-precipitated Al, showing the lowest (0.6%) and the greatest (65%) \( \frac{\text{Fe(II)}_{\text{total}}}{\text{Fe(III)}_{\text{total}}} \) ratio in the 0- and 75-AFH sample after 168 h of bioreduction (Figure 3.3). This result demonstrates that co-precipitated Al enhanced bioreducibility of Fe(III)-hydroxides.

**Iron K-Edge XANES Spectroscopy of Bioreduced AFH**

Normalized Fe K-edge XANES spectra for AFH samples exposed anaerobically with CN32 for 0.5 h and 168 h are shown in Figure 3.4a and 3.4b. Individual spectra comprise three distinct regions: a pre-edge peak around 7114 eV, a sharp increase in absorption between 7124 to 7128 eV (absorption edge), and a white line (WL) around 7132 eV, which are attributed to an electron transition from 1s to 3d-4p mixing, 4s-4p mixing, and 4p molecular orbitals, respectively (Chen et al., 2002; Waychunas et al., 1983).

Compared to the consistent energy of absorption edge (7128 eV) for non-inoculated samples (Figure A3.2 in Appendix), absorption-edge energy for bioreduced AFH samples decreased disproportionately with increasing Al mol% and bioreduction period (Figure 3.4a and b). In general terms, transition to lower energy for absorption edge suggests a decrease in oxidation state, thereby such energy shift has been reported as one of the indicators to determine changes in Fe valence (Waychunas et al., 1983). For example, the absorption edge for Fe(II) minerals occur at \(~2.4\) eV lower than that for Fe(III) minerals (Waychunas et al., 1983; Wilke et al., 2001). In our bioreduced samples, a correlation \( r^2 = 0.91, p = 0.05 \) was found between absorption-edge energy and the proportion of total Fe(II) to total Fe in
samples for XAS analysis, wherein, the lowest absorption-edge energy, 7124 eV, was found in 50- and 75-AFH samples that were bioreduced for 168 h (Figure A3.3 in Appendix).

**Local Coordination Structure of Bioreduced AFH**

The $k^3$-weighted Fe K-edge EXAFS spectra for AFH samples incubated with CN32 for 0.5 h and 168 h are shown in Appendix Figure A3.4a and A3.4b. For both times, spectral features in k-space were almost identical across all samples, except for the lower peak intensity at 6.5, 7.5, and 8.5 Å$^{-1}$ coming with increasing Al proportion. The Fourier transform magnitude of EXAFS spectra for 0.5-h- and 168-h-bioreduced samples shows two peaks centered at ~1.5 and ~2.8 Å (Figure 3.5a and 3.5b). EXAFS fitting results for the first-shell Fe-O and the higher-shell Fe-Fe coordination are tabulated and in Table 3.1 and 3.2. Comparisons of our fitting models and data are shown in Appendix Figures A3.5 and A3.6 for samples that were bioreduced for 0.5 and 168 h, wherein goodness-of-fit parameters ($R$-factors) were between 2.3 and 2.6% (Table 3.1). Also, contributions of individual backscattering paths to the EXAFS spectra are shown for the 0-AFH samples in Appendix Figures A3.7 and A3.8. The following discussions focus on trends in first-shell Fe-O and higher-shell Fe-Fe coordination in relation to bioreduction of our AFH samples.

**The First Coordination Shell**

For 0.5-h-bioreduced samples, the first peak at magnitude portion of EXAFS spectra (Figure 3.5a) was determined as the Fe-O coordination at ~1.99 Å with coordination numbers (CN) ranging from 5.4±0.6 to 5.9±0.6 (Table 3.1). The Fe-O paths with similar distances
were also found in the 168-h-bioreduced samples, but the CN decreased systematically from 5.7 ± 0.7 to 4.5 ± 0.6 as Al increased up to 75 mol%. Because samples for EXAFS analysis were prepared as moist pastes, each single Fe atom might be coordinated with 6 oxygen atoms that could be satisfied by pervasive water molecules regardless of surface defects (Manceau and Gates, 1997). However, the CN (within uncertainties) for the 168-h-bioreduced samples containing Al ≥ 20 mol% is less than 6, the typical CN for the first-shell Fe-O. Although Michel et al. (2007) suggested a 20% content of tetrahedral Fe(III) occurring in two-line ferrihydrite, Manceau (2009; 2010) has argued that tetrahedral Fe violated Pauling’s 2nd and 3rd rules. The reduction in the first-peak amplitude, thereby, is expected to lie in the partially destructive interference between individual Fe-O wave functions, which is presumably due to the wide range of first-shell Fe-O distances (1.62-2.48 Å) split with the increasing ratio of surface to bulk Fe atoms (Manceau, 2009; Manceau and Gates, 1997; Voegelin et al., 2010). Such increasing proportion of surface-located FeO₆ may be caused by a reduction in the domain size of Fe-hydroxide or by the sorption of Fe(II). The latter point was evident by the proportion of total Fe(II) to total Fe in 168-h-bioreduced samples for XAS analysis, which increased from 4 to 15% while Al co-precipitation increased from 20 to 75 mol% (Figure A3.3 in Appendix). To account for a first-shell Fe-O coordination that according to science should be 6, an alternative EXAFS fitting that guessed mean-square displacement of interatomic distance (σ²) with a fixed CN at 6 for the first-shell Fe-O path was conducted (Table A3.2 in Appendix). Given that σ² describes a distribution of interatomic distances within a single shell (Kelly et al., 2008), the increasing σ² for 168-h-
bioreduced co-precipitates suggested a relatively wider range of Fe-O distance for samples with higher Al mol\%, agreeing with the discussion mentioned earlier.

Recalling macroscopic results of the 168-h-bioreduced samples with 75 mol\% of Al, which has the lowest CN for the first-shell Fe-O path, 15\% of total Fe(II) was found in total Fe (Figure A3.3 in Appendix). Therefore, the longer Fe(II)-O distance [2.11 Å, (Thoral et al., 2005)] compared with the Fe(III)-O path [1.99 Å, (Michel et al., 2007)] may partially contribute to the destructive interference in the first-shell Fe-O wave functions. Reasons that the longer Fe-O distance corresponding to the Fe(II)-O path was not determined from EXAFS analysis (Table 3.1) could be: (1) EXAFS recognizes ≤ 10\% of what XANES spectrum can detect from absorption edge itself (Kelly et al., 2008), (2) the 15 \% of Fe(II) / Fe(III) (Figure 3.2 and 3.3) in residual 75-AFH only contribute 0.018 Å difference in Fe-O distance [15\% × (2.11 Å - 1.99 Å) = 0.018 Å, given that 2.11 and 1.99 Å are the distance for Fe(II)-O and Fe(III)-O path (Michel et al., 2007; Thoral et al., 2005)]; the differentiation goes beyond EXFAS fitting limitation, and (3) the Fe(II)-O distance differs from that obtained from crystallographic data [2.11 Å (Thoral et al., 2005)] because the symmetry of surface-located Fe(II)O$_6$ may be altered by the bonding with water molecules (Manceau and Gates, 1997).

**The Higher Coordination Shell: Fe-Fe Paths**

In the magnitude part of EXAFS spectra, particularly striking was the significantly enhanced second peak at 2.7 Å for the 0-AFH sample after 0.5 h of bioreduction (Figure 3.5a). The higher-shell coordination for both 0.5-h- and 168-h-bioreduced samples was fit
using two Fe-Fe paths and three Fe-O paths as shown in Table 3.2 and Appendix Table A3.3 and A3.4. In co-precipitates after 0.5 h of bioreduction, interatomic distances of Fe-Fe1 and Fe-Fe2 paths were determined as ~3.05 and ~3.43 Å, consistent with edge- and corner-sharing linkages of FeO\textsubscript{6} (Chapter 2), wherein CN for Fe-Fe1 path decreased linearly from 3.2 ± 0.6 to 1.0 ± 0.6 as Al co-precipitation increased up to 75 mol% ($r^2 = 0.94$, $p = 0.05$) (Table 3.2, Appendix Figure A3.9). For nano-scale AFH samples (~2 nm (Michel et al., 2007)), this trend corresponds with decreasing Fe-hydroxide domain size along edge-sharing FeO\textsubscript{6} (Chapter 2). By contrast, no systematic change was observed in the CN of corner-sharing FeO\textsubscript{6} designated as Fe-Fe2 (Table 3.2).

Except for pure Fe-hydroxide (0-AFH), the structural parameters of 0.5-h-bioreduced AFH were generally similar as that of non-inoculated samples (Table 3.1, 3.2 and Table 2.1 in Chapter 2). In 0-AFH, a decrease in $\sigma^2$ for Fe-Fe subshells from 0.012 ± 0.002 for non-inoculated to 0.007 ± 0.001 Å\textsuperscript{2} for 0.5-h-bioreduced sample was found (Table 2.1 in Chapter 2, Table 3.2). This decreasing $\sigma^2$ implies an increase in structural ordering and is presumably the reason causing the particularly enhanced peak at 2.7 Å in the spectrum of 0-AFH in that $\sigma^2$ is a modeling parameter that affects the amplitude of EXAFS data (Kelly et al., 2008). Lacking the disturbance of Al co-precipitation, a treatment of 0.5-h microbial exposure appears to induce a greater structural ordering in Fe domains. However, the degree of such structural development is not to the level forming a well-crystalline Fe-(hydr)oxide as shown in the XRD patterns (Figure A3.10 in Appendix). Compared to the Fourier transformed magnitude parts of well-crystalline Fe-(hydr)oxides (Figure A3.11 in Appendix), 0-AFH
apparently lacked the growth in corner-sharing linkages, agreeing with the relatively lower CN (0.7 ± 0.4) for the Fe-Fe2 path (Table 3.1).

As the 0-AFH sample was bioreduced for 168 h, a progressive change shown as an emerging shoulder occurred at the second peak of the magnitude portion of EXAFS data (Figure 3.5b). With 0-AFH as an exception, structural parameters between 0.5-h- and 168-h-bioreduced samples were nearly identical within uncertainties (Table 3.2). For 0-AFH, the spectral feature of an emerging shoulder at 3.2 Å along with the stronger peak at 2.7 Å (Figure 3.5b) implied the recovery of corner-sharing linkage, concurring with the increasing CN at Fe-Fe2 path from 0.7 ± 0.4 to 2.1 ± 0.9 as bioreduction period extended from 0.5 h to 168 h (Table 3.2). The growth in corner-sharing FeO₆ rendered EXAFS spectrum of 0-AFH analogous to that of six-line ferrihydrite (Toner et al., 2009), and could be treated as the continuation of microbial-structural development (Kukkadapu et al., 2003). Such change in molecular configurations, however, was not observed in XRD patterns (Figure A3.10 in Appendix).

**DISCUSSION**

**Effects of Al Co-Precipitation on Bioreduction of Fe-Hydroxides**

The presence of Al co-precipitated with Fe-hydroxide at 20 to 75 mol% strongly increased the extent of AFH bioreduction by CN32 and influenced the partitioning of biogenic Fe(II) to aqueous and solid phases. In our co-precipitated systems, Al presented as discrete phases rather than the monolayer associated with Fe-hydroxides (Chapter 2).
Although these Al polymers tended to locate on near-surfaces of co-precipitates (Chapter 2), the inhibition in Fe(III) bioreducibility as a consequence of blocked electron transfer caused by such Al was not found. That is, in contrast to what Murray and Hesterberg (2006) reported: Al cations essentially decreased the abiotic Fe(III) reduction from ferrihydrite/boehmite mixtures by passivating ferrihydrite surfaces.Apparently, such suppressive effect seemed to be offset in dissimilatory microbial reduction of Al/Fe-hydroxide co-precipitates.

As discussed in Chapter 2, Al co-precipitates disturbed structural development of Fe-hydroxides, exhibiting a shorter-range structural ordering and a smaller Fe domain size with increasing Al mol%. Such perturbation may lead to more susceptible particle surface and an increasing surface area in Fe domains, and thereby, may improve the accessibility for electron transfer. Compared with samples containing Al co-precipitates, pure Fe-hydroxides (0-AFH) showed a more organized arrangement in FeO₆ linkages after a 0.5-h exposure to CN32 (Table 3.2). After 168 h of bioreduction, additional structural development in Fe domains was found in 0-AFH but not in other AFH samples (Table 3.2). Although we have insufficient information to understand the overall mechanisms, the microbial-induced structural development could partially account for this structural changes due to a strong force of interaction between Fe-(hydr)oxides and *Shewanella* species (Lower et al., 2001). It was found that nano-scale ferrihydrite can cluster on and bind with surfaces of *Shewanella putrefaciens* within a 0.5-h exposure (Glasauer et al., 2001). However, the presence of Al co-precipitates limited such microbial-induced polymerization in Fe domains as indicated by our EXAFS analysis. Here, Al acted like a structurally incompatible cation that inhibits polymerization of Fe-(hydr)oxides (Schwertmann and Fischer, 1973). Particles with shorter-
range structural order are more available to microbes and thereby may essentially increase the Fe(III) bioreducibility (Lovley and Phillips, 1986).

Catalyzed via microbes, the reduction of Fe(III)-hydroxides is a complex process that is sensitive to numerous factors such as mineral phases and bacterial metabolism. For example, the blocked electron transfer caused by Al in abiotic Fe(III) reduction as reported by Murray and Hesterberg (2006) could be possibly overcome if *Shewanella* species are involved. For those Fe(III)-hydroxides that are not in direct contact with cells, *Shewanella* species is able to promote bioreduction by producing chelators that solubilize Fe(III) and/or by releasing extracellular electron-shuttling compounds that can expedite electron transfer (Lovley et al., 2004; Newman and Kolter, 2000; von Canstein et al., 2008). A combination of bacterial characteristics and Al effects on Fe polymerization may explain the intriguing reduction behavior for AFH samples.

**CONCLUSIONS**

Co-precipitated Al was shown to enhance dissimilatory microbial reduction of short-range order Fe(III)-hydroxides by optimizing bioavailability of Fe domains. Accompanying the presence of *Shewanella putrefaciens* CN32, a continuous structural development was found in the pure Fe-hydroxide systems but not in samples containing Al co-precipitates. This observation suggested that Al polymers appeared to stabilize Fe domains against the microbial-induced structural development. Much of previous work examining Al effects on Fe(III) bioreduction has focused on the extent and biomineralization. This study
complemented the mechanical mechanisms at a molecular level. Presented as a co-precipitated phase, Al influenced Fe bioreduction in both abiotic and biotic conditions, which are equally significant while predicting the biogeochemical behavior of dissimilatory metal reduction in natural environments.
REFERENCES


Table 3.1. Structural parameters for first-shell Fe-O contributions derived from Fe K-edge EXAFS analysis for Al/Fe-hydroxide co-precipitates with 0, 20, 50, and 75 mol% of total metal as Al (0-, 20-, 50-, 75-AFH) incubated with *S. putrefaciens* CN32 for 0.5 and 168 h.a

<table>
<thead>
<tr>
<th>Sample</th>
<th>Path</th>
<th>R (Å)b</th>
<th>CNc</th>
<th>σ² (Å²)d</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 h</td>
<td>Fe-O1</td>
<td>2.00 (0.006)</td>
<td>5.5 (0.5)</td>
<td>0.010 (0.001)</td>
</tr>
<tr>
<td>20-AFH</td>
<td>Fe-O1</td>
<td>1.99 (0.008)</td>
<td>5.9 (0.6)</td>
<td>0.012 (0.001)</td>
</tr>
<tr>
<td>50-AFH</td>
<td>Fe-O1</td>
<td>2.00 (0.009)</td>
<td>5.4 (0.6)</td>
<td>0.012 (0.001)</td>
</tr>
<tr>
<td>75-AFH</td>
<td>Fe-O1</td>
<td>1.98 (0.007)</td>
<td>5.4 (0.6)</td>
<td>0.012 (0.001)</td>
</tr>
<tr>
<td>168 h</td>
<td>Fe-O1</td>
<td>2.00 (0.008)</td>
<td>5.7 (0.7)</td>
<td>0.011 (0.002)</td>
</tr>
<tr>
<td>20-AFH</td>
<td>Fe-O1</td>
<td>2.00 (0.007)</td>
<td>5.2 (0.5)</td>
<td>0.011 (0.001)</td>
</tr>
<tr>
<td>50-AFH</td>
<td>Fe-O1</td>
<td>1.98 (0.009)</td>
<td>4.9 (0.5)</td>
<td>0.011 (0.001)</td>
</tr>
<tr>
<td>75-AFH</td>
<td>Fe-O1</td>
<td>1.98 (0.013)</td>
<td>4.5 (0.6)</td>
<td>0.011 (0.001)</td>
</tr>
</tbody>
</table>

a The amplitude reduction factor ($S_0^2$), transferred from first-shell fitting of hematite, was fixed at 0.83 ± 0.18 for all EXAFS fitting. All samples were fit using one energy shift parameter: ΔE = -1.45 ± 0.50 and -0.12 ± 0.53 for 0.5-h- and 168-h-bioreduced samples. The normalized sum of the squared residuals of the overall fit ($R = \sum(data-fit)^2/\sum data^2$) was 2.3% and 2.6% for fits of 0.5-h- and 168-h-bioreduced samples. See Table A3.3 and A3.4 in Appendix for all structural parameters.
b Interatomic distance.
c Degeneracy (coordination number, CN).
d Mean-square displacement of interatomic distance ($\sigma^2$). One $\sigma^2$ value was shared across samples of 20-, 50-, and 75-AFH to decrease the number of fitting parameters (Chapter 2).
Table 3.2. Structural parameters for Fe-Fe contributions derived from Fe K-edge EXAFS analysis for Al/Fe-hydroxide co-precipitates with 0, 20, 50, and 75 mol% of total metal as Al (0-, 20-, 50-, 75-AFH) incubated with *S. putrefaciens* CN32 for 0.5 and 168 h.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Path</th>
<th>R (Å)</th>
<th>CN</th>
<th>σ² (Å²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 h</td>
<td>0-AFH</td>
<td>Fe-Fe1</td>
<td>3.06 (0.006)</td>
<td>3.2 (0.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fe-Fe2</td>
<td>3.44 (0.006)</td>
<td>0.7 (0.4)</td>
</tr>
<tr>
<td></td>
<td>20-AFH</td>
<td>Fe-Fe1</td>
<td>3.05 (0.016)</td>
<td>2.1 (0.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fe-Fe2</td>
<td>3.43 (0.016)</td>
<td>1.5 (1.0)</td>
</tr>
<tr>
<td>168 h</td>
<td>0-AFH</td>
<td>Fe-Fe1</td>
<td>3.05 (0.016)</td>
<td>2.1 (0.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fe-Fe2</td>
<td>3.43 (0.016)</td>
<td>1.5 (1.0)</td>
</tr>
<tr>
<td>168 h</td>
<td>20-AFH</td>
<td>Fe-Fe1</td>
<td>3.06 (0.011)</td>
<td>2.9 (0.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fe-Fe2</td>
<td>3.44 (0.011)</td>
<td>2.6 (1.1)</td>
</tr>
<tr>
<td></td>
<td>50-AFH</td>
<td>Fe-Fe1</td>
<td>3.04 (0.020)</td>
<td>1.6 (0.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fe-Fe2</td>
<td>3.42 (0.020)</td>
<td>2.4 (1.2)</td>
</tr>
<tr>
<td></td>
<td>75-AFH</td>
<td>Fe-Fe1</td>
<td>3.04 (0.054)</td>
<td>1.1 (1.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fe-Fe2</td>
<td>3.42 (0.054)</td>
<td>1.5 (1.7)</td>
</tr>
</tbody>
</table>

*a* General fitting information was listed in the footnote of Table 3.1. See Table A3.3 and A3.4 in Appendix for all structural parameters.

*b* One common ΔR values was designated to the group of Fe-Fe paths.

*c* One common σ² value was designated to the group of Fe-Fe paths, which was shared across samples of 20-, 50-, and 75-AFH to decrease the number of fitting parameters (Chapter 2).
Figure 3.1. Concentration of (a) total (0.5 M HCl-extractable) and (b) dissolved Fe(II) produced during bioreduction of Al/Fe-hydroxide co-precipitates with 0, 10, 20, 50, 75, and 100 mol% of total metal as Al (0-, 10-, 20-, 50-, 75-, 100-AFH). Results are shown as average of triplicate measurements with error bars indicating one standard deviation. For control samples devoid of CN 32 bacteria, total and dissolved Fe(II) concentrations were always ≤ 0.1 mmol L\(^{-1}\) and undetectable with detection limit of 0.8 mg L\(^{-1}\). The initial (time 0) pH was 5.5, and the average final pH value is shown next to each treatment. The inset in Figure 3.1b shows an expanded view of dissolved Fe(II) produced from 0-AFH.
Figure 3.2. Soild phase Fe(II) observed in bioreduction of Al/Fe-hydroxide co-precipitates with 0, 10, 20, 50, 75, and 100 mol% of total metal as Al (0-, 10-, 20-, 50-, 75-, 100-AFH), represented as proportion of total Fe(II) production. Results are shown as average of triplicate measurements. Error bars indicate one standard deviation.
Figure 3.3. Trends in total (0.5 M HCl-extractable) Fe(II) produced from microbial reduction of Al/Fe-hydroxide co-precipitates with 0, 10, 20, 50, 75, and 100 mol\% of total metal as Al (0-, 10-, 20-, 50-, 75-, 100-AFH), represented as proportion of total Fe added. Results are shown as average of triplicate measurements. Error bars indicate one standard deviation. Total Fe(II) was generally under 0.1 mmol L\(^{-1}\) for control samples devoid of CN 32 bacteria.
Figure 3.4. Normalized Fe K-edge XANES spectra for Al/Fe-hydroxide co-precipitates with 0, 20, 50, and 75 mol% of total metal as Al (0-, 20-, 50-, 75-AFH) incubated with *S. putrefaciens* CN32 for (a) 0.5 h and (b) 168 h.
Figure 3.5. The Fourier transformed magnitude of Al/Fe-hydroxide co-precipitates with 0, 20, 50, and 75 mol% of total metal as Al (0-, 20-, 50-, 75-AFH) incubated with *S. putrefaciens* CN32 for (a) 0.5 and (b) 168 h.
Table A3.1. Bioreduction of ferrihydrite conducted at various pH and buffer solutions.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Microbe</th>
<th>pH buffer</th>
<th>Initial pH</th>
<th>Final pH</th>
<th>% of total Fe(III) reduced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fredrickson et al., 1998</td>
<td><em>Shewanella putrefaciens</em> CN32</td>
<td>PIPES&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.8</td>
<td>7.1</td>
<td>11% (20 d)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NaHCO₃</td>
<td>6.8</td>
<td>7.1</td>
<td>33% (20 d)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Zachara et al., 1998</td>
<td><em>Shewanella putrefaciens</em> CN32</td>
<td>NaHCO₃</td>
<td>~7.0</td>
<td>N/A</td>
<td>13% (39 d)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Urrutia et al., 1998</td>
<td><em>Shewanella alga</em> BrY</td>
<td>PIPES&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.8</td>
<td>N/A</td>
<td>60% (5 d)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Zachara et al., 2002</td>
<td><em>Shewanella putrefaciens</em> CN32</td>
<td>MES&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.8</td>
<td>7.2</td>
<td>40% (5 d)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Campbell et al., 2006</td>
<td><em>Shewanella ANA-3</em> WT</td>
<td>NaHCO₃</td>
<td>6.8</td>
<td>8.3</td>
<td>33% (5 d)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NaHCO₃</td>
<td>8.0</td>
<td>N/A</td>
<td>18% (5 d)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> 1,4-piperazinediethanesulfonic acid.

<sup>b</sup> 2-(N-Morpholino)ethanesulfonic acid.

<sup>c</sup> After this period of bioreduction.
Table A3.2. Structural parameters for first-shell Fe-O contributions derived from Fe K-edge EXAFS analysis using alternative model with fixed coordination number of six for Al/Fe-hydroxide co-precipitates with 0, 20, 50, and 75 mol% of total metal as Al (0-, 20-, 50-, 75-AFH) incubated with *S. putrefaciens* CN32 for 0.5 and 168 h.\(^a\)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Path</th>
<th>(R) (Å)(^b)</th>
<th>CN(^c)</th>
<th>(\sigma^2) (Å(^2))(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 h</td>
<td>0-AFH</td>
<td>Fe-O1</td>
<td>2.00 (0.006)</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>20-AFH</td>
<td>Fe-O1</td>
<td>1.99 (0.008)</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>50-AFH</td>
<td>Fe-O1</td>
<td>2.00 (0.009)</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>75-AFH</td>
<td>Fe-O1</td>
<td>1.98 (0.008)</td>
<td>6</td>
</tr>
<tr>
<td>168 h</td>
<td>0-AFH</td>
<td>Fe-O1</td>
<td>2.00 (0.007)</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>20-AFH</td>
<td>Fe-O1</td>
<td>2.00 (0.007)</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>50-AFH</td>
<td>Fe-O1</td>
<td>1.98 (0.009)</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>75-AFH</td>
<td>Fe-O1</td>
<td>1.99 (0.014)</td>
<td>6</td>
</tr>
</tbody>
</table>

\(^a\) The amplitude reduction factor \((S_0^2)\), transferred from first-shell fitting of hematite, was fixed at 0.83 ± 0.18 for all EXAFS fitting. All samples were fit using one energy shift parameter: \(\Delta E = -1.70 ± 0.49\) and \(-0.49 ± 0.55\) for 0.5-h- and 168-h-bioreduced samples.

The normalized sum of the squared residuals of the overall fit \((R = \sum(\text{data-fit})^2/\sum\text{data}^2)\) was 2.4% and 2.9% for fits of 0.5-h- and 168-h-bioreduced samples.

\(^b\) Interatomic distance.

\(^c\) Degeneracy (coordination number, CN), which was fixed at 6 for all samples.

\(^d\) Mean-square displacement of interatomic distance \((\sigma^2)\).
Table A3.3. Structure parameters derived from Fe K-edge EXAFS spectra analysis for Al/Fe-hydroxide co-precipitates with 0, 20, 50, and 75 mol% of total metal as Al (0-, 20-, 50-, 75-AFH) incubated with *S. putrefaciens* CN32 for 0.5 h.\(^a\)

<table>
<thead>
<tr>
<th>Path</th>
<th>R (Å)</th>
<th>CN(^c)</th>
<th>(\sigma^2 (Å^2))(^e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-AFH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe-O1</td>
<td>2.00 (0.006)</td>
<td>5.5 (0.5)</td>
<td>0.010 (0.001)</td>
</tr>
<tr>
<td>Fe-Fe1</td>
<td>3.06 (0.006)</td>
<td>3.2 (0.6)</td>
<td>0.007 (0.001)</td>
</tr>
<tr>
<td>Fe-Fe2</td>
<td>3.44 (0.006)</td>
<td>0.7 (0.4)</td>
<td>-</td>
</tr>
<tr>
<td>Fe-O2</td>
<td>3.42 (0.006)</td>
<td>d</td>
<td>0.010 (0.003)</td>
</tr>
<tr>
<td>Fe-O3</td>
<td>3.63 (0.006)</td>
<td>d</td>
<td>-</td>
</tr>
<tr>
<td>Fe-O4</td>
<td>3.81 (0.006)</td>
<td>d</td>
<td>-</td>
</tr>
<tr>
<td>20-AFH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe-O1</td>
<td>1.99 (0.008)</td>
<td>5.9 (0.6)</td>
<td>0.012 (0.001)(^f)</td>
</tr>
<tr>
<td>Fe-Fe1</td>
<td>3.05 (0.016)</td>
<td>2.1 (0.8)</td>
<td>0.011 (0.002)(^f)</td>
</tr>
<tr>
<td>Fe-Fe2</td>
<td>3.43 (0.016)</td>
<td>1.5 (1.0)</td>
<td>-</td>
</tr>
<tr>
<td>Fe-O2</td>
<td>3.42 (0.016)</td>
<td>d</td>
<td>0.015 (0.005)(^f)</td>
</tr>
<tr>
<td>Fe-O3</td>
<td>3.62 (0.016)</td>
<td>d</td>
<td>-</td>
</tr>
<tr>
<td>Fe-O4</td>
<td>3.80 (0.016)</td>
<td>d</td>
<td>-</td>
</tr>
<tr>
<td>50-AFH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe-O1</td>
<td>2.00 (0.009)</td>
<td>5.4 (0.6)</td>
<td>0.012 (0.001)(^f)</td>
</tr>
<tr>
<td>Fe-Fe1</td>
<td>3.05 (0.019)</td>
<td>1.6 (0.8)</td>
<td>0.011 (0.002)(^f)</td>
</tr>
<tr>
<td>Fe-Fe2</td>
<td>3.43 (0.019)</td>
<td>1.6 (1.0)</td>
<td>-</td>
</tr>
<tr>
<td>Fe-O2</td>
<td>3.42 (0.019)</td>
<td>d</td>
<td>0.015 (0.005)(^f)</td>
</tr>
<tr>
<td>Fe-O3</td>
<td>3.62 (0.019)</td>
<td>d</td>
<td>-</td>
</tr>
<tr>
<td>Fe-O4</td>
<td>3.80 (0.019)</td>
<td>d</td>
<td>-</td>
</tr>
<tr>
<td>75-AFH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe-O1</td>
<td>1.98 (0.007)</td>
<td>5.4 (0.6)</td>
<td>0.012 (0.001)(^f)</td>
</tr>
<tr>
<td>Fe-Fe1</td>
<td>3.04 (0.020)</td>
<td>1.0 (0.6)</td>
<td>0.011 (0.002)(^f)</td>
</tr>
<tr>
<td>Fe-Fe2</td>
<td>3.42 (0.020)</td>
<td>1.4 (0.8)</td>
<td>-</td>
</tr>
<tr>
<td>Fe-O2</td>
<td>3.40 (0.020)</td>
<td>d</td>
<td>0.015 (0.005)(^f)</td>
</tr>
<tr>
<td>Fe-O3</td>
<td>3.61 (0.020)</td>
<td>d</td>
<td>-</td>
</tr>
<tr>
<td>Fe-O4</td>
<td>3.79 (0.020)</td>
<td>d</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^a\)The amplitude reduction factor (\(S_0^2\)), transferred from first-shell fitting of hematite, was fixed at 0.83 ± 0.18 for all EXAFS fitting. All samples were fit using one energy shift parameter: \(\Delta E = -1.45 ± 0.50\). The normalized sum of the squared residuals of the overall fit \((R = \sum(data-fit)^2/\sum data^2)\) was 2.3% for all fits.  
\(^b\)Interatomic distance. The \(\Delta R\) value for the group of higher-shell Fe-O paths was fit using the same floated parameter designated to the group of Fe-Fe paths.  
\(^c\)Degeneracy (coordination number, CN).  
\(^d\)CN for higher-shell Fe-O paths were constrained along with that of edge-sharing (Fe1) and corner-sharing (Fe2) octahedra (Chapter 2).  
\(^e\)Mean-square displacement of interatomic distance (\(\sigma^2\)). One \(\sigma^2\) value was designated for each group of paths.  
\(^f\)One floated \(\sigma^2\) value was shared for the first-shell Fe-O, Fe-Fe, and higher-shell Fe-O atom pairs across samples of 20-, 50-, and 75-AFH to decrease the number of fitting parameters (Chapter 2).
Table A3.4. Structure parameters derived from Fe K-edge EXAFS spectra analysis for Al/Fe-hydroxide co-precipitates with 0, 20, 50, and 75 mol% of total metal as Al (0-, 20-, 50-, 75-AFH) incubated with *S. putrefaciens* CN32 for 168 h.\(^a\)

<table>
<thead>
<tr>
<th>Path</th>
<th>R (Å)(^b)</th>
<th>CN(^c)</th>
<th>(\sigma^2 (\text{Å}^2))(^e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-AFH</td>
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<td></td>
</tr>
<tr>
<td>Fe-O1</td>
<td>2.00 (0.008)</td>
<td>5.7 (0.7)</td>
<td>0.011 (0.002)</td>
</tr>
<tr>
<td>Fe-Fe1</td>
<td>3.05 (0.009)</td>
<td>2.9 (0.8)</td>
<td>0.009 (0.002)</td>
</tr>
<tr>
<td>Fe-Fe2</td>
<td>3.42 (0.009)</td>
<td>2.1 (0.9)</td>
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<td>Fe-O2</td>
<td>3.41 (0.009)</td>
<td>5.7 (0.7)</td>
<td>0.010 (0.002)</td>
</tr>
<tr>
<td>Fe-O3</td>
<td>3.62 (0.009)</td>
<td>5.7 (0.7)</td>
<td></td>
</tr>
<tr>
<td>Fe-O4</td>
<td>3.80 (0.009)</td>
<td>5.7 (0.7)</td>
<td></td>
</tr>
<tr>
<td>20-AFH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe-O1</td>
<td>2.00 (0.007)</td>
<td>5.2 (0.5)</td>
<td>0.011 (0.001)(^f)</td>
</tr>
<tr>
<td>Fe-Fe1</td>
<td>3.06 (0.011)</td>
<td>2.9 (0.9)</td>
<td>0.013 (0.003)(^f)</td>
</tr>
<tr>
<td>Fe-Fe2</td>
<td>3.44 (0.011)</td>
<td>2.6 (1.1)</td>
<td></td>
</tr>
<tr>
<td>Fe-O2</td>
<td>3.43 (0.011)</td>
<td>5.2 (0.5)</td>
<td>0.016 (0.002)(^f)</td>
</tr>
<tr>
<td>Fe-O3</td>
<td>3.64 (0.011)</td>
<td>5.2 (0.5)</td>
<td></td>
</tr>
<tr>
<td>Fe-O4</td>
<td>3.81 (0.011)</td>
<td>5.2 (0.5)</td>
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</tr>
<tr>
<td>50-AFH</td>
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<td></td>
</tr>
<tr>
<td>Fe-O1</td>
<td>1.98 (0.009)</td>
<td>4.9 (0.5)</td>
<td>0.011 (0.001)(^f)</td>
</tr>
<tr>
<td>Fe-Fe1</td>
<td>3.04 (0.020)</td>
<td>1.6 (0.8)</td>
<td>0.013 (0.003)(^f)</td>
</tr>
<tr>
<td>Fe-Fe2</td>
<td>3.42 (0.020)</td>
<td>2.4 (1.2)</td>
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</tr>
<tr>
<td>Fe-O2</td>
<td>3.41 (0.020)</td>
<td>5.2 (0.5)</td>
<td>0.016 (0.002)(^f)</td>
</tr>
<tr>
<td>Fe-O3</td>
<td>3.62 (0.020)</td>
<td>5.2 (0.5)</td>
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</tr>
<tr>
<td>Fe-O4</td>
<td>3.79 (0.020)</td>
<td>5.2 (0.5)</td>
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</tr>
<tr>
<td>75-AFH</td>
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<td></td>
</tr>
<tr>
<td>Fe-O1</td>
<td>1.98 (0.013)</td>
<td>4.5 (0.6)</td>
<td>0.011 (0.001)(^f)</td>
</tr>
<tr>
<td>Fe-Fe1</td>
<td>3.04 (0.054)</td>
<td>1.1 (1.1)</td>
<td>0.013 (0.003)(^f)</td>
</tr>
<tr>
<td>Fe-Fe2</td>
<td>3.42 (0.054)</td>
<td>1.5 (1.7)</td>
<td></td>
</tr>
<tr>
<td>Fe-O2</td>
<td>3.40 (0.054)</td>
<td>5.2 (0.5)</td>
<td>0.016 (0.002)(^f)</td>
</tr>
<tr>
<td>Fe-O3</td>
<td>3.62 (0.054)</td>
<td>5.2 (0.5)</td>
<td></td>
</tr>
<tr>
<td>Fe-O4</td>
<td>3.79 (0.054)</td>
<td>5.2 (0.5)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)The amplitude reduction factor \((S_0^2)\), transferred from first-shell fitting of hematite, was fixed at 0.83 ± 0.18 for all EXAFS fitting. All samples were fit using one energy shift parameter: -0.12 ± 0.53. The normalized sum of the squared residuals of the overall fit \((R = \sum\text{(data-fit)}^2/\sum\text{data}^2)\) was 2.6% for all fits.  
\(^b\)Interatomic distance. The ΔR value for the group of higher-shell Fe-O paths was fit using the same floated parameter designated to the group of Fe-Fe paths.  
\(^c\)Degeneracy (coordination number, CN).  
\(^d\)CN for higher-shell Fe-O paths were constrained along with that of edge-sharing (Fe1) and corner-sharing (Fe2) octahedra (Chapter 2). \(\text{CNO}_2 = 1/4*\text{CNFe}_2; \text{CNO}_3 = 1/4*\text{CNFe}_2 + 1/2*\text{CNFe}_1; \text{CNO}_4 = 1/2*\text{CNFe}_2 + \text{CNFe}_1\).  
\(^e\)Mean-square displacement of interatomic distance \((\sigma^2)\). One \(\sigma^2\) value was designated for each group of paths.  
\(^f\)One floated \(\sigma^2\) value was shared for the first-shell Fe-O, Fe-Fe, and higher-shell Fe-O atom pairs across samples of 20-, 50-, and 75-AFH to decrease the number of fitting parameters (Chapter 2).
Figure A3.1. Correlation between concentration of total (0.5 M-extractable) Fe(II) and pH after suspensions of Al/Fe-hydroxide co-precipitates with 0, 10, 20, 50, 75, and 100 mol% of total metal as Al (0-, 10-, 20-, 50-, 75-, 100-AFH) were bioreduced with *S. putrefaciens* CN32 for 168 h.
Figure A3.2. Normalized Fe K-edge XANES spectra for Al/Fe-hydroxide co-precipitates with 0, 20, 50, and 75 mol% of total metal as Al (0-, 20-, 50-, 75-AFH) without S. putrefaciens CN32 inoculation.
Figure A3.3. Linear regression between energy of absorption edge at Fe K-edge x-ray absorption spectrum and the proportion of total Fe(II) to total Fe in samples for XAS analysis after suspensions of Al/Fe-hydroxide co-precipitates with 0, 20, 50, and 75 mol% of total metal as Al (0-, 20-, 50-, 75-AFH) were bioreduced with *S. putrefaciens* CN32 for 168 h.

\[
y = -0.33x + 7128.28 \\
\]

\[
r^2 = 0.91^* \\
\]
Figure A3.4. The Fe K-edge EXAFS spectra of Al/Fe-hydroxide co-precipitates with 0, 20, 50, and 75 mol% of total metal as Al (0-, 20-, 50-, 75-AFH) incubated with *S. putrefaciens* CN32 for (a) 0.5 and (b) 168 h.
Figure A3.5. Real part of Fourier transformed EXAFS spectra for experiment data (open circles) and fitting results (solid lines) of Al/Fe-hydroxide co-precipitates with 0, 20, 50, and 75 mol% of total metal as Al (0-, 20-, 50-, 75-AFH) incubated with *S. putrefaciens* CN32 for 0.5 h.
Figure A3.6. Real part of Fourier transformed EXAFS spectra for experiment data (open circles) and fitting results (solid lines) of Al/Fe-hydroxide co-precipitates with 0, 20, 50, and 75 mol% of total metal as Al (0-, 20-, 50-, 75-AFH) incubated with *S. putrefaciens* CN32 for 168 h.
Figure A3.7. Real part of Fourier transformed EXAFS spectra for experiment data (open circles) and fitting results (solid lines) of 0-AFH (ferrihydrite) incubated with *S. putrefaciens* CN32 for 0.5 h. Scattering paths fitted using a structural model for two line-ferrihydrite [2-nm domain - (Michel et al., 2007)] illustrate the contribution from absorber atoms (Fe) to surrounding atoms (O and Fe).
Figure A3.8. Real part of Fourier transformed EXAFS spectra for experiment data (open circles) and fitting results (solid lines) of 0-AFH (ferrihydrite) incubated with *S. putrefaciens* CN32 for 168 h. Scattering paths fitted using a structural model for two line-ferrihydrite [2-nm domain - (Michel et al., 2007)] illustrate the contribution from absorber atoms (Fe) to surrounding atoms (O and Fe).
Figure A3.9. Linear regression between coordination number of edge-sharing FeO$_6$ linkages and Al proportion in Al/Fe-hydroxide co-precipitates after samples were bioreduced with *S. putrefaciens* CN32 for 0.5 h.

\[ y = -0.03 x + 2.97 \]
\[ r^2 = 0.94^* \]
Figure A3.10. X-ray diffraction patterns of ferrihydrite incubated with *S. putrefaciens* CN32 for 0.5 h (0-AFH/bioreduction/0.5 h), 168 h (0-AFH/bioreduction/168 h), and the control sample devoid of CN 32 bacteria (0-AFH). Data were collected at Beamline of BL13A1 at National Synchrotron Radiation Research Center (NSRRC), Hsinchu, Taiwan, using bicron diffractometer with a wavelength of 1.0269 Å adjusted with Si(111) monochromator. Each spectrum shows two broad peaks centered at 2.6 and 1.5 Å, agreeing with those of two-line ferrihydrite (Schwertmann and Cornell, 2000).
Figure A3.11. The Fourier transformed magnitude of goethite, lepidocrocite, hematite (Hesterberg, unpublished data), Fe-hydroxide (0-AFH – Chapter 2), and Fe-hydroxides incubated with *S. putrefaciens* CN32 for 0.5 h and for 168 h.
CHAPTER 4
Phosphate Distribution during Microbial Reduction of Non-Crystalline Al/Fe-
Hydroxide Co-Precipitates

ABSTRACT

Orthophosphate (PO$_4$) may be either dissolved or sorbed by anaerobic soils as
affected by the complex assemblages in natural environments. Our objective in this study
was to determine the re-distribution of PO$_4$ between solid phases and aqueous solution during
microbial reduction of phosphated Al(III)/Fe(III)-hydroxide co-precipitates. Phosphate was
sorbed at concentrations from 42 to 138 mmol mol$^{-1}$ Al+Fe on co-precipitates containing 0 to
75 mol% of Al/(Al+Fe) molar ratios. As promoted by *Shewanella putrefaciens* CN32,
bioreduction of phosphated co-precipitates was influenced by not only Al proportion but also
sorbed PO$_4$ concentration. An enhanced Fe bioreduction with increasing sorbed PO$_4$
concentration was found in co-precipitates containing $\leq$ 20 mol% of Al, wherein PO$_4$ tended
to stabilize Fe domains against microbial-induced structural development. Over the course of
bioreduction, no PO$_4$ dissolution was detected. Results of x-ray absorption near edge
structure (XANES) spectroscopy indicated Fe[(II)/(III)]-PO$_4$ bonding that increased with
increasing extent of Fe bioreduction generally dominated solid-phase PO$_4$ in co-precipitates
with $\leq$ 20 mol% of Al. Alternatively, a preferential PO$_4$ bonding to Al accounted for the PO$_4$
retention in samples containing $\geq$ 50 mol% of Al. This study provided a sense of
biogeochemical intricacies should be considered while estimating PO₄ destiny in natural environments.

**INTRODUCTION**

Phosphorus (P), a dynamic and biologically active element contained in various compounds, may be chemically or enzymatically hydrolyzed to orthophosphate (PO₄), which is the most bioavailable form of P. The environmental concern of PO₄ lies in the discharge of PO₄ to water systems and the subsequent oxygen depletion, causing the problem of eutrophication (Correll, 1998; Foy, 2005). By shielding the downstream aquatic ecosystem from excess fertilizer or manure PO₄ exported from uplands, soils play a vital role in the protection of water quality. Poorly-crystalline Al- and Fe-(hydr)oxides have been proposed to be mainly responsible for PO₄ retention in soils (Beauchemin and Simard, 1999). For such sorbed PO₄, bonding distribution between Al vs. Fe is meaningful as it potentially influences the mobility of PO₄, particularly under reducing conditions.

In anaerobic soils and sediments, PO₄ dissolution is considered to be induced by the reductive dissolution of Fe(III)-bound PO₄ (Holford and Patrick, 1981; Patrick and Khalid, 1974; Phillips, 1998; Sallade and Sims, 1997; Scharer et al., 2009; Willett, 1989; Young and Ross, 2001). Subsequent to Fe(III) reduction, however, diminishing accumulation of soluble PO₄, resorption on freshly precipitated amorphous Fe(OH)₂ material and/or Fe(II)/Fe(III)-hydroxide compounds, was also reported by several studies (Holford and Patrick, 1981; Patrick and Khalid, 1974; Phillips, 1998; Willett, 1985; Willett, 1989). Alternatively, the PO₄
retention capacity for redox-inactive Al-(hydr)oxides rarely changed with the fluctuation in redox potential, indicating the significance of Al-(hydr)oxides as PO$_4$ sorbents in reducing environments (Axt and Walbridge, 1999; Darke and Walbridge, 2000; Richardson, 1985).

Interactions between organic and inorganic constituents in natural environments often influence the dynamic cycling of elements. In systems of dissimilatory Fe(III) bioreduction, added PO$_4$ affected the reducibility, stability, and mineralization of Fe(III)-(hydr)oxides. During Fe(III) bioreduction, concentrations of added PO$_4$ decreased as dissolved Fe(II) was generated (Urrutia et al., 1998; Zachara et al., 1998), which was attributed to precipitation/resorption with Fe(II) and/or to assimilation by bacterial cells. Also, precipitation of vivianite [Fe$_3$(PO$_4$)$_2$$\cdot$8H$_2$O] depleted the amount of Fe(II) and thereby limited the emergence of magnetite (Fe$_3$O$_4$) (Fredrickson et al., 2001; Urrutia et al., 1998). Moreover, added PO$_4$ was expected to alter surface reactivity of Fe(III)-hydroxides as proposed from a linear decrease in Fe(III) bioreducibility with increasing PO$_4$ surface coverage, (Borch et al., 2007). However, an enhancement in the extent of Fe(III) bioreduction caused by the addition of PO$_4$ was also documented in previous studies (Fredrickson et al., 1998; Kukkadapu et al., 2004). Lacking molecular-scale evidence, many unknowns are left in the interactions between PO$_4$ and Fe-(hydr)oxide bioreduction.

Our objective in this study was to determine the re-distribution of PO$_4$ between solid phases and aqueous solution during microbial reduction of phosphated Al(III)/Fe(III)-hydroxide co-precipitates (AFH). We quantified dissolved PO$_4$ and the relative PO$_4$ distribution on residual solid phases. This study was conducted using a combination of macroscopic reduction experiments with synchrotron-based spectroscopic analyses.
Phosphorus speciation could be achieved using P K-edge x-ray absorption near edge structure (XANES) spectroscopy. For example, Fe(III)-bound PO$_4$ can be differentiated from that associated with Al(III) or organic compounds using the discernible spectral features (Beauchemin et al., 2003; Hesterberg et al., 1999; Khare et al., 2004). The contribution of this research is to provide fundamental knowledge for PO$_4$ destiny in complex biogeochemical systems among oxyanions, minerals, and microbes.

**MATERIALS AND METHODS**

**Synthesis of Non-Crystalline Al/Fe-Hydroxide Co-Precipitates (AFH)**

Details in AFH synthesis has been described in Chapter 2. Briefly, AFH samples were freshly precipitated by hydrolyzing solution mixtures of Fe(NO$_3$)$_3$·9H$_2$O and Al(NO$_3$)$_3$·9H$_2$O with Al/(Al+Fe) molar ratios of 0, 0.2, 0.5, 0.75, and 1.0. The co-precipitates were hereafter named as 0-, 20-, 50-, 75-, and 100-AFH.

**Phosphate Sorption Isotherms**

Phosphated AFH samples used in bioreduction experiments were prepared via sorption isotherms with PO$_4$ concentrations ranging from 42 to 175 mmol mol$^{-1}$ Al+Fe on individual AFH suspensions. The procedure is analogous to that reported in Chapter 2, except that the equilibrium pH of 5.5 and the background electrolyte of 0.05 M KCl were used here. Isotherm experiments with higher PO$_4$ addition were conducted to determine the maximum PO$_4$ sorption capacity.
Bioreduction Experiments

Procedures for bioreduction of phosphated AFH are comparable to that described in Chapter 3 with the only difference being that AFH samples used here contained sorbed PO\textsubscript{4} at concentrations of 42 to 138 mmol mol\textsuperscript{-1} Al+Fe. Bioreduction of suspensions that comprised 50 mmol Al+Fe L\textsuperscript{-1} as phosphated AFH samples, 0.025 M sodium formate, 0.05 M 2-(N-Morpholino)ethanesulfonic acid potassium salt (MES), 0.05 M KCl, and \(~3\times10^{11}\) cells L\textsuperscript{-1} of inoculum was promoted by *Shewanella putrefaciens* CN 32 (CN32) for up to 168 h. Periodically, an aliquot of suspension was subsampled for the measurements of pH, cell density, and Fe(II) concentration. Based on the fact that short-range order Al- and Fe-hydroxides are rapidly dissolved by oxalate (Jackson et al., 1986), the cell density was determined by adding 6 mL of 0.2-μm filtered 0.2 M ammonium oxalate to 1-mL portion of suspensions (Roden and Zachara, 1996) and measuring absorbance at 420 nm. Biogenic Fe(II) was measured as dissolved Fe(II), the portion passing through a 0.2-μm membrane filter, and as total Fe(II), the portion extracted using 0.5 M HCl. After 168 h of bioreduction, solids of selected samples separated upon 0.2-μm membrane filters from suspensions were collected for x-ray absorption spectroscopy (XAS) analyses including P K-edge XANES and Fe K-edge extended x-ray absorption fine structure (EXAFS) spectroscopy to determine the distribution of PO\textsubscript{4} bonding and the local coordination of Fe in bioreduced samples. Sample handling was done in a glove box under an N\textsubscript{2(g)} atmosphere.
Phosphorus K-Edge XANES Data Collection and Analysis

Phosphorus K-edge XANES spectra for phosphated AFH samples and that incubated with CN32 were collected at Beamline BL16A1 at the National Synchrotron Radiation Research Center, Hsinchu, Taiwan. Details on beamline equipment, data collection, spectral background removal, and linear combination fitting (LCF) were described previously in Chapter 2. Standards used for LCF of bioreduced phosphated AFH samples include sorbed PO$_4$ associated with Fe(III) and Al(III), strengite, short-range order Fe(III)-PO$_4$ minerals, and sorbed PO$_4$ on CN32.

Iron K-Edge XAS Data Collection and Analysis

The procedures for Fe K-edge XAS data collection and analysis, and EXAFS fitting details for bioreduced phosphated AFH samples are analogous to that reported in Chapter 2, except that the spectra were acquired at Beamline BL16A1 at the National Synchrotron Radiation Research Center, Hsinchu, Taiwan.

RESULTS and DISCUSSION

Phosphate Sorption and Distribution on AFH at pH 5.5

Phosphate sorption isotherms on individual AFH samples at pH 5.5 and 0.05 M KCl background electrolyte were fitted with Freundlich models (Sposito, 1984) (Figure A4.1 in Appendix). The maximum PO$_4$ level sorbed on co-precipitates increased disproportionally
from 273 to 475 mmol mol\(^{-1}\) Al+Fe (2970 to 5680 mmol kg\(^{-1}\)) as Al increased from 0 to 100 mol\%, which is up to 1.2- to 1.5-fold greater than that found at pH 6.0 (Chapter 2).

Normalized P K-edge XANES spectra for PO\(_4\) sorbed on co-precipitates at pH 5.5 as shown in Figure A4.2 in Appendix were characterized by a sharp-increasing white line (WL) around 2152 eV. At the pre-WL region from 2144-2150 eV, spectral intensity was independent of sorbed PO\(_4\) concentrations (Figure 4.1a-c) but decreased systematically with increasing Al co-precipitation (Figure 4.1d). Relative PO\(_4\) distribution between Fe(III) and Al(III) on AFH samples was quantified using LCF across the pre-WL region with end members of PO\(_4\) sorbed on ferrihydrite and on non-crystalline Al-hydroxide (Khare et al., 2004) (Table 4.1, Figure 4.2). Compared with reference lines, which were calculated based on molar ratios of co-precipitated metals (Chapter 2), the relatively lower amount of PO\(_4\) associated with Fe on samples of 20- and 50-AFH (Figure 4.2) suggested a preferential bonding of PO\(_4\) to Al. For the 75-AFH sample, however, the coincidence between reference line and linear regression fit (Figure 4.2) implied non-preferential PO\(_4\) binding to either Al or Fe. These trends are comparable with that found at pH 6.0, wherein surface chemical composition, degree of polymerization, and Fe domain size of co-precipitates were concluded as factors governing PO\(_4\) distribution (Chapter 2).

**Bioreduction of Phosphated AFH**

The extent of total (0.5 M HCl-extractable) Fe(II) generated during bioreduction of phosphated AFH samples varied with sorbed PO\(_4\) concentration and proportion of Al co-precipitation (Figure 4.3). As PO\(_4\) was sorbed at ~43 mmol P mol\(^{-1}\) Al+Fe (Figure 4.3a),
trends in bioreducibility across all AFH samples mimicked that found in samples devoid of PO₄ sorption (Chapter 3). There, an enhancement in biogenic Fe(II) was observed as Al co-precipitation increased from 0 to 50 mol%, but a further increase in Al to 75 mol% incrementally decreased Fe(II) production (Figure 4.3a). This trend, however, was altered by increasing PO₄ addition, which enhanced Fe(II) production between samples of 0- and 20-AFH, but had no effect on Fe reduction in co-precipitates with ≥ 50 mol% of Al (Figure 4.3b-d). In the 0-AFH sample, increasing PO₄ concentration from 46 to 138 mmol mol⁻¹ Al+Fe resulted in an exponential increase (up to 30 fold) in Fe(II) production (Figure 4.4). Alternatively, a 2-fold but linear increase in Fe(II) production was found in the 20-AFH sample as sorbed PO₄ increased from 44 to 132 mmol mol⁻¹ Al+Fe (Figure 4.4). For samples of 50- and 75-AFH, however, bioreducibility remained relatively constant, irrespective of PO₄ concentration (Figure 4.4). For reference, bioreduction of Fe-hydroxides acts as a proton-consuming reaction.

\[
2\text{Fe(OH)}_3(s) + \text{HCOO}^-_{(aq)} + 4\text{H}^+_{(aq)} = 2 \text{Fe}^{2+}_{(aq)} + \text{HCO}_3^-_{(aq)} + 5 \text{H}_2\text{O}(l) \tag{1}
\]

Therefore, final pH values for AFH samples varied with total Fe(II) production, which drifted from an initial pH of 5.5 to pH 6.9 in this study (Figure 4.3).

**Iron Octahedral Linkages in Bioreduced Phosphated AFH**

Iron octahedral linkages for the phosphated 0-AFH sample with 0.5 h of bioreduction were fit at distances of 3.05 ± 0.017 Å (Fe-Fe1) and 3.43 ± 0.017 Å (Fe-Fe2) with coordination numbers (CN) of 1.8 ± 0.5 and 0.6 ± 0.6, respectively (Table 4.2, Figure 4.5, Figure A4.3 in Appendix). Mean-square displacement of interatomic distance (σ²) for this fit
was \(0.011 \pm 0.002 \text{ Å}^2\) (Table 4.2). Compared with the non-phosphated 0-AFH sample after 0.5 h of bioreduction, wherein CN for the 3.05-Å Fe-Fe path was \(3.2 \pm 0.6\), and \(\sigma^2\) was \(0.007 \pm 0.001 \text{ Å}^2\) (Table 3.1 in Chapter 3), fewer Fe-O₆ linkages and greater \(\sigma^2\) value both suggested that the presence of PO₄ caused a greater structural disorder or less polymerization in Fe domains. Structural changes caused by PO₄ mimics that induced by the Al co-precipitation as described in Chapter 3. We reported that Al co-precipitation enhanced bioreducibility for AFH samples by hindering microbial-induced structural development of Fe-O₆ linkages. For samples containing 50 and 75 mol% of Al, bulk Al clusters presumably caused a greater decrease in degree of polymerization than PO₄ effects, resulting in no relationship between their bioreducibility and sorbed PO₄ concentration (Figure 4.4).

**Phosphate Distribution on AFH after 168 H of Bioreduction**

Phosphate is a normal constituent for the basal media used to culture and to study Fe(III) dissimilatory reductive mechanisms (Lovley and Phillips, 1986; Roden and Zachara, 1996; Zachara et al., 1998). However, discussion pertaining to PO₄ distribution during Fe(III) bioreduction is lacking. In our study, PO₄ was applied via sorption on AFH samples prior to bioreduction process. For the given PO₄ sorption from 42 to 138 mmol mol⁻¹ Al+Fe, \(\leq 0.1\%\) of added PO₄ was detected in aqueous phases. During bioreduction experiments, no dissolved PO₄ was detected using the molybdate colorimetric method with 1-cm cell path length. Phosphate, therefore, was expected to mainly stay in solid phases, either as the original sorbed species, by incorporation into residual solids as biomineralization products, or associated with bacterial cells. In the following discussion, P K-edge XANES spectroscopy
was used to determine the distribution of solid phase PO$_4$, particularly for the relative association between Fe and Al.

Normalized P K-edge XANES spectra for 168-h-bioreduced AFH samples were characterized by a strong WL peak, analogous to that of non-inoculated samples (Figure 4.6 and A4.2 in Appendix). Derivative XANES spectra in Figure 4.7 show that the WL energy for 168-h-bioreduced 0- and 20-AFH samples with sorbed PO$_4$ of 138 and 132 mmol mol$^{-1}$ Al+Fe was approximately 0.16- and 0.11-eV higher than that for the rest of the samples. In Fe(II)/Fe(III) mixtures, such increasing WL energy may be caused by the increasing amount of Fe(II)-PO$_4$ bonding (Hesterberg et al. – unpublished data), that is, implying parts of PO$_4$ were retained by Fe(II) in these samples. Phosphate distribution on residual bioreduced solids was further determined by focusing on the pre-WL features that are more distinguishable for Fe(III)-bonded PO$_4$ vs. Al(III)-bonded PO$_4$ or other PO$_4$ species (Hesterberg, 2010; Khare et al., 2005). In contrast to the substantially independent relationship between pre-WL intensity and sorbed PO$_4$ concentration in non-inoculated samples (Figure 4.1), samples of 168-h-bioreduced 0- and 20-AFH showed a disproportional increase in pre-WL intensity with increasing PO$_4$ sorption (Figure 4.8a-b). This trend, however, was not found as Al proportion $\geq$ 50 mol% (Figure 4.8c-d). For a given PO$_4$ concentration, pre-WL intensity essentially decreased as Al increased up to 75 mol%, but that for 100-AFH was the same as 75-AFH (Figure 4.8e-f). Assuming that pre-WL intensity is proportional to the number of Fe(III)-PO$_4$ bonds (Khare et al., 2007), PO$_4$ bonding was changed during bioreduction in relation to sorbed PO$_4$ concentration and proportions of Al co-precipitation.
Standards used in LCF to probe the relative PO₄ distribution are shown in Figure 4.9 along with a representative sample, 168-h-bioreduced 0-AFH with 138 mmol mol⁻¹ Al+Fe of PO₄. Spectra for PO₄ sorbed on ferrihydrite and on non-crystalline Al-hydroxide (0- and 100-AFH/P) have similar WL feature but opposite pre-WL intensity, which is pronounced for 0-AFH but minimal for 100-AFH. Compared to PO₄ sorbed on ferrihydrite, spectra for short-range order Fe(III)-PO₄ [Fe(III)-PO₄] and strengite were characterized by shorter WL peaks and more distinct pre-WL features. For PO₄ sorbed on CN32 [1000 mmol kg⁻¹, (CN32/P)], a weak pre-WL feature and a relatively broader WL peak was observed. Unfortunately, LCF is limited to differentiate bonding between Al(III)-PO₄ and Fe(II)-PO₄ because of their featureless and undistinguished pre-WL region (Hesterberg, 2010). For such minerals, only the relative degree of crystallinity can be determined via the changes in WL intensity (Hesterberg, 2010; Hesterberg et al., 1999).

Results of LCF show that spectra for 168-h-bioreduced 0-AFH samples with ≤ 92 mmol P mol⁻¹ Al+Fe cannot be fit by any standard combinations but the sole spectrum of PO₄ sorbed on ferrihydrite. However, as sorbed PO₄ increased up to 138 mmol mol⁻¹ Al+Fe, 50% of PO₄ on 168-h-bioreduced 0-AFH was fit as short-range order Fe(III)-PO₄ (Table 4.3), which was used to estimate the increasing amount of Fe(III)-PO₄ bonds as implied by the enhanced pre-WL peak (Khare et al., 2007). We do not think that crystalline Fe(III)-PO₄ compounds formed in our bioreduction samples because we found no change in WL intensity (Figure 4.6). A relatively lower WL intensity and a greater full width at half-maximum height (fwhm) is expected as PO₄ bonding changes from adsorption to precipitation (Khare et al., 2005). In LCF of bioreduced 0-AFH samples, the fitting program did not accept the
spectral combination of Fe(III)-associated sorbed PO₄ with Fe(II)-PO₄ as being low-end standards. Lacking Fe(II)-PO₄ as an end member (Table 4.3), quantification of PO₄ species may be limited. The contribution of featureless pre-WL region given by Fe(II)-PO₄ bonds in bioreduced 0-AFH sample with 138 mmol P mol⁻¹ Al+Fe (Figure 4.7) may be compensated by decreasing the proportion of Fe(III)-PO₄ together with increasing the proportion of Fe(III)-associated sorbed PO₄. Hence, P XANES analysis failed to quantify PO₄ species, but suggested a general increase in the number of Fe(III)-PO₄ bonds. A similar circumstance was found in the 20-AFH sample with PO₄ concentration of 132 mmol mol⁻¹ Al+Fe, wherein 7% of the solid phase PO₄ was fit as short-range order Fe(III)-PO₄, and the rest was recognized as Fe(III)-associated sorbed PO₄ (Table 4.3). Due to the interference of Fe(II)-PO₄ bonding (Figure 4.7), LCF only signaled a overall increase in the amount of Fe(III)-associated PO₄ bonding rather than giving an accurate quantification of individual species. For example, the given 93% of Fe(III)-associated, sorbed PO₄ in the phosphated (132 mmol P mol⁻¹ Al+Fe) 20-AFH sample (Table 4.3) seemed to exceed the sorption capacity that residual Fe(III) can provide.

Except for the 20-AFH with 132 mmol P mol⁻¹ Al+Fe, pre-WL regions for samples containing 20-75 mol% of Al and various PO₄ addition (42-131 mmol P mol⁻¹ Al+Fe) were only fit by the combination of Fe(III)- and Al(III)-associated sorbed PO₄ (Table 4.3). Here, Fe(II)-PO₄ was excluded as an end member in LCF because the absence of Fe(II)-PO₄ bonds was suggested by indiscernible changes in WL energy for these samples (Figure 4.7). It is conceivable that plenty of Al(III) and Fe(III) (≥ 82 mol% - Figure 4.3) in residual solids of these samples served as the major sorbents for PO₄ retention against the formation of Fe(II)
PO₄ complexes. Results of LCF for bioreduced 20-AFH samples showed that the proportion of PO₄ associated with Fe(III) relative to Al(III) generally increased with increasing sorbed PO₄ concentration (Table 4.3, Figure 4.10). Compared to the reference line, the upward deviation of linear regression fit for 20-AFH samples indicated that a preferential PO₄ bonding to Fe(III) appeared as PO₄ concentration > 88 mmol mol⁻¹ Al+Fe (Figure 4.10). Phosphate on bioreduced 50-AFH sampled, however, was generally dominated by an preferential bonding to Al(III), as suggested by the linear regression fit that deviated downward from the reference line. This trend of PO₄ distribution was indistinguishable from that of non-inoculated 50-AFH samples (p = 0.05) (Figure 4.2, 4.10). For the bioreduced 75-AFH, the proportion of PO₄ associated with Al(III) ranged from 74 - 83% of sorbed PO₄ and suggested preferential bonding to Al(III). However, this result contrasts to the non-preferential binding of PO₄ to either Fe(III) or Al(III) examined prior to the bioreduction (Figure 4.2, 4.10).

Taken together, Fe(III) bioreduction in AFH samples did not trigger sorbed PO₄ dissolution, even though PO₄ was bonded with Fe(III). However, Fe(III) may be partially reduced from surface sites without bonded PO₄ because the maximum concentration for PO₄ addition (~130 mmol mol⁻¹ Al+Fe) only occupied ≤ 51% of PO₄ sorption capacity for AFH samples. The striking changes in PO₄ distribution (Figure 4.2, Figure 4.10) suggested that Fe(III) bioreduction potentially induced the re-distribution of PO₄ between Fe or Al sites. Either Fe or Al dominated the PO₄ retention, which is mainly controlled by availability of binding sites on the residual hydroxide domains rather than redox-activity of elements. In the Fe(III)-dominated systems (Al ≤ 20 mol%), PO₄ retention, even with enhanced Fe(III)
bioreducibility, was mainly dominated by Fe[(II)/(III)]-PO$_4$ bonding. As Al(III) became dominated (Al ≥ 50 mol%), PO$_4$ was largely bound to the available Al(III) sites.

**CONCLUSIONS**

In this study, PO$_4$ sorbed on co-precipitated Al/Fe-hydroxides resulted in a facilitated dissimilatory Fe(III) reduction by stabilizing Fe domains against polymerization. During dissimilatory Fe(III) reduction, such PO$_4$ was mainly bound by the residual Fe[(II)/(III)] or Al(III), and microbial uptake was not detected. The distribution of PO$_4$ binding between Fe and Al in hydroxides depended on the element proportion in AFH samples. As a result, the hypothesized reductive dissolution of PO$_4$ was impeded. Knowledge of PO$_4$ distribution between redox-active Fe and redox-inactive Al is useful for interpreting the PO$_4$ dissolution under reducing soil conditions, and for estimating PO$_4$ mobility in wetland soils. However, mechanisms of PO$_4$ dissolution along with Fe(III)-(hydr)oxide bioreduction in such multi-component systems was not supported by our data in this research.
REFERENCES


Table 4.1. Phosphate associated with Fe(III) and Al(III) at pH 5.5 on Al/Fe-hydroxide coprecipitates with 20, 50, and 75 mol% of total metal as Al (20-, 50-, 75-AFH) calculated using linear combination fitting of normalized P K-XANES spectra.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sorbed PO$_4$ (mmol mol$^{-1}$ Al+Fe)</th>
<th>P-ferrihydrite (%)$^a$</th>
<th>P-non-xl Al-hydroxide (%)$^a$</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-AFH</td>
<td>44</td>
<td>69 ± 0.6</td>
<td>31 ± 0.6</td>
<td>0.0011</td>
</tr>
<tr>
<td></td>
<td>66</td>
<td>71 ± 0.7</td>
<td>29 ± 0.7</td>
<td>0.0019</td>
</tr>
<tr>
<td></td>
<td>88</td>
<td>69 ± 0.6</td>
<td>31 ± 0.6</td>
<td>0.0013</td>
</tr>
<tr>
<td></td>
<td>132</td>
<td>73 ± 0.8</td>
<td>27 ± 0.8</td>
<td>0.0022</td>
</tr>
<tr>
<td></td>
<td>175</td>
<td>74 ± 0.8</td>
<td>26 ± 0.8</td>
<td>0.0021</td>
</tr>
<tr>
<td>50-AFH</td>
<td>44</td>
<td>36 ± 1.0</td>
<td>64 ± 1.0</td>
<td>0.0033</td>
</tr>
<tr>
<td></td>
<td>65</td>
<td>47 ± 0.8</td>
<td>53 ± 0.8</td>
<td>0.0024</td>
</tr>
<tr>
<td></td>
<td>87</td>
<td>37 ± 0.7</td>
<td>63 ± 0.7</td>
<td>0.0017</td>
</tr>
<tr>
<td></td>
<td>131</td>
<td>36 ± 0.9</td>
<td>64 ± 0.9</td>
<td>0.0025</td>
</tr>
<tr>
<td></td>
<td>174</td>
<td>44 ± 0.7</td>
<td>56 ± 0.7</td>
<td>0.0018</td>
</tr>
<tr>
<td>75-AFH</td>
<td>42</td>
<td>33 ± 0.5</td>
<td>67 ± 0.5</td>
<td>0.0010</td>
</tr>
<tr>
<td></td>
<td>63</td>
<td>20 ± 0.6</td>
<td>80 ± 0.6</td>
<td>0.0014</td>
</tr>
<tr>
<td></td>
<td>84</td>
<td>18 ± 1.0</td>
<td>82 ± 1.0</td>
<td>0.0035</td>
</tr>
<tr>
<td></td>
<td>126</td>
<td>25 ± 0.9</td>
<td>75 ± 0.9</td>
<td>0.0026</td>
</tr>
<tr>
<td></td>
<td>168</td>
<td>26 ± 0.5</td>
<td>74 ± 0.5</td>
<td>0.0008</td>
</tr>
</tbody>
</table>

$^a$ Standards using for linear combination fitting were PO$_4$ sorbed on ferrihydrite (P-ferrihydrite) with 181 mmol P mol$^{-1}$ Fe and on non-crystalline Al-hydroxide (P-non-xl Al-hydroxide) with 166 mmol P mol$^{-1}$ Al reacted at pH 5.5 and 0.05 M KCl background electrolyte. Deviations (±) represent uncertainties calculated by Athena from the linear combination fitting.
Table 4.2. Structure parameters for Fe-Fe contributions obtained from Fe K-edge EXAFS spectra analysis for phosphated (92 mmol P mol\(^{-1}\) Al+Fe) Al/Fe-hydroxide co-precipitates containing 0 mol% of total metal as Al (0-AFH – Fe-hydroxide) incubated with \textit{S. putrefaciens} CN32 for 0.5 h.\(^a\)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Path</th>
<th>R (\text{Å})(^b)</th>
<th>CN(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-AFH</td>
<td>Fe-Fe1</td>
<td>3.05 (0.017)</td>
<td>1.8 (0.5)</td>
</tr>
<tr>
<td></td>
<td>Fe-Fe2</td>
<td>3.43 (0.017)</td>
<td>0.6 (0.6)</td>
</tr>
</tbody>
</table>

\(^a\) The amplitude reduction factor (S\(_0^2\)), transferred from first-shell fitting of hematite, was fixed at 0.83 ± 0.18. Energy shift parameter was fit as (\(\Delta E = 3.15 \pm 0.56\) eV). Besides two Fe-Fe paths, spectrum was fit using one Fe-O (Fe-O1) and three Fe-O (Fe-O2, -O3, and -O4) paths for the first- and higher-shell peaks. Mean-square displacement of interatomic distance (\(\sigma^2\)) was designated for each group of paths, which is 0.010 (0.002) Å\(^2\), 0.011 (0.002) Å\(^2\), and 0.013 (0.002) Å\(^2\) for the first-shell Fe-O, Fe-Fe, and higher-shell Fe-O paths. The normalized sum of the squared residuals of the fit (\(R = \sum\text{(data-fit)}^2/\sum\text{data}^2\)) was 2.7\%.

\(^b\) Interatomic distance. Path distances of Fe-O1, -O2, -O3, and -O4 were fit as 2.00 (0.008), 3.41 (0.017), 3.62 (0.017), and 3.80 (0.017) Å.

\(^c\) Degeneracy (coordination number, CN). CN for Fe-O1 was fit as 4.7 (0.3). CNs for Fe-O2, -O3, and -O4 were constrained along with that of edge-sharing (Fe1) and corner-sharing (Fe2) octahedra. CNO\(_2\) = 1/4*CNFe\(_2\); CNO\(_3\) = 1/4*CNFe\(_2\) + 1/2*CNFe\(_1\); CNO\(_4\) = 1/2*CNFe\(_2\) + CNFe\(_1\) (Chapter 2).
Table 4.3. Phosphate distribution calculated using linear combination fitting of normalized P K-edge XANES spectra for phosphated Al/Fe-hydroxide co-precipitates with 0, 20, 50, and 75 mol% of total metal as Al (0-, 20-, 50-, 75-AFH) incubated with *S. putrefaciens* CN32 for 168 h.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sorbed PO$_4$ (mmol mol$^{-1}$ Al+Fe)</th>
<th>P-ferrihydrite (%)$^a$</th>
<th>P-non-xl Al-hydroxide (%)$^a$</th>
<th>Fe(III)-PO$_4$ (%)$^a$</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-AFH$^b$</td>
<td>138</td>
<td>50 ± 5.2</td>
<td>50 ± 5.2</td>
<td>0.0328</td>
<td></td>
</tr>
<tr>
<td>20-AFH</td>
<td>44</td>
<td>61 ± 1.3</td>
<td>39 ± 1.3</td>
<td>0.0057</td>
<td></td>
</tr>
<tr>
<td></td>
<td>66</td>
<td>78 ± 1.0</td>
<td>22 ± 1.0</td>
<td>0.0027</td>
<td></td>
</tr>
<tr>
<td></td>
<td>88</td>
<td>75 ± 0.8</td>
<td>25 ± 0.8</td>
<td>0.0021</td>
<td></td>
</tr>
<tr>
<td></td>
<td>132</td>
<td>93 ± 5.9</td>
<td>7 ± 5.9</td>
<td>0.0401</td>
<td></td>
</tr>
<tr>
<td>50-AFH</td>
<td>44</td>
<td>40 ± 0.9</td>
<td>60 ± 0.9</td>
<td>0.0023</td>
<td></td>
</tr>
<tr>
<td></td>
<td>65</td>
<td>55 ± 0.8</td>
<td>45 ± 0.8</td>
<td>0.0018</td>
<td></td>
</tr>
<tr>
<td></td>
<td>87</td>
<td>43 ± 0.9</td>
<td>57 ± 0.9</td>
<td>0.0024</td>
<td></td>
</tr>
<tr>
<td></td>
<td>131</td>
<td>39 ± 0.6</td>
<td>61 ± 0.6</td>
<td>0.0009</td>
<td></td>
</tr>
<tr>
<td>75-AFH</td>
<td>42</td>
<td>18 ± 0.9</td>
<td>82 ± 0.9</td>
<td>0.0023</td>
<td></td>
</tr>
<tr>
<td></td>
<td>63</td>
<td>26 ± 1.3</td>
<td>74 ± 1.3</td>
<td>0.0051</td>
<td></td>
</tr>
<tr>
<td></td>
<td>84</td>
<td>18 ± 0.9</td>
<td>82 ± 0.9</td>
<td>0.0025</td>
<td></td>
</tr>
<tr>
<td></td>
<td>126</td>
<td>17 ± 0.5</td>
<td>83 ± 0.5</td>
<td>0.0007</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Standards using for linear combination fitting were PO$_4$ sorbed on ferrihydrite (P-ferrihydrite) with 181 mmol P mol$^{-1}$ Fe and on non-crystalline Al-hydroxide (P-non-xl Al-hydroxide) with 166 mmol P mol$^{-1}$ Al reacted at pH 5.5 and 0.05 M KCl background electrolyte. Fe(III)-PO$_4$ represents as the standard for short-range order Fe(III)-PO$_4$ mineral. Deviations (±) represent uncertainties calculated by Athena from the linear combination fitting.

$^b$ Samples of bioreduced 0-AFH with sorbed PO$_4$ concentrations ranging over from 46 to 92 mmol mol$^{-1}$ Fe only fit with the single standard of P-ferrihydrite.
Figure 4.1. Pre-white line region of normalized XANES spectra for PO₄ sorbed at various concentrations (in mmol P mol⁻¹ Al+Fe as denoted by numbers in the legend) on Al/Fe-hydroxide co-precipitates with Al contents of (a) 20 (20-AFH), (b) 50 (50-AFH), and (c) 75 (75-AFH) mol% of total metal concentration; and for (d) PO₄ sorbed at ~172 mmol P mol⁻¹ Al+Fe across all samples (175, 174, and 168 mmol P mol⁻¹ Al+Fe for 20-, 50-, and 75-AFH). Spectra were compared with end members of PO₄ sorbed on ferrihydrite (0-AFH) with 181 mmol P mol⁻¹ Fe and on non-crystalline Al-hydroxide (100-AFH) with 166 mmol P mol⁻¹ Al.
Figure 4.2. Phosphate distribution associated with Fe(III) vs. Al(III) on Al/Fe-hydroxide co-precipitates with 20, 50, and 75 mol% of total metal as Al (20-, 50-, 75-AFH) determined by XANES analysis as the function of total sorbed PO$_4$. Dashed lines are reference lines (RL) that represent the expected Fe(III)-associated PO$_4$ if PO$_4$ was distributed with Fe(III) and Al(III) in proportion to their concentrations in the co-precipitates (non-preference). Linear regression fits to the data are shown as solid lines.
Figure 4.3. Concentration of total (0.5 M HCl-extractable) Fe(II) produced from phosphated Al/Fe-hydroxide co-precipitates with 0, 20, 50, and 75 mol% of total metal as Al (0-, 20-, 50-, 75-AFH) incubated with *S. putrefaciens* CN32. The concentration of sorbed PO₄ is denoted in the legend. Sorbed PO₄ concentrations for the 0-AFH samples are 46, 67, 92, and 138 mmol mol⁻¹ Al+Fe. See Table 4.1 for PO₄ sorption concentration on 20-, 50-, and 75-AFH samples. The initial (time 0) pH was 5.5, and the average final pH is shown next to each treatment. Biogenic Fe(II) was undetectable with detection limit of 0.8 mg L⁻¹ for phosphated 100-AFH samples.
Figure 4.4. Concentration of total (0.5 M HCl-extractable) Fe(II) production collected after 168 h of bioreduction for Al/Fe-hydroxide co-precipitates with 0, 20, 50, and 75 mol% of total metal as Al (0-, 20-, 50-, 75-AFH) as a function of sorbed PO₄ concentration.
Figure 4.5. Fourier transformed magnitude for phosphated (92 mmol P mol\(^{-1}\) Al+Fe) Al/Fe-hydroxide co-precipitates containing 0 mol% of total metal as Al (0-AFH – Fe-hydroxide) incubated with \textit{S. putrefaciens} CN32 for 0.5 h.
Figure 4.6. Normalized P K-edge XANES spectra for PO$_4$ sorbed at various concentrations (in mmol P mol$^{-1}$ Al+Fe shown as numbers in the legend) on Al/Fe-hydroxide co-precipitates with 0, 20, 50, 75, and 100 mol% of total metal as Al (0-, 20-, 50-, 75-, 100-AFH) incubated with *S. putrefaciens* CN32 for 168 h. Sorbed PO$_4$ concentrations are 46, 67, 92, and 138 mmol mol$^{-1}$ Al+Fe on 0-AFH; 42, 63, 84, 125 mmol mol$^{-1}$ Al+Fe on 100-AFH. See Table 4.1 for PO$_4$ sorption concentration on 20-, 50-, and 75-AFH samples.
Figure 4.7. Derivative P K-edge XANES spectra for PO₄ sorbed at various concentrations (in mmol P mol⁻¹ Al+Fe shown as numbers in the legend; see caption of Figure 4.6 for the exact sorption concentration) on Al/Fe-hydroxide co-precipitates with 0, 20, 50, 75, and 100 mol% of total metal as Al (0-, 20-, 50-, 75-, 100-AFH) incubated with S. putrefaciens CN32 for 168 h.
Figure 4.8. Pre-white line region of normalized XANES spectra for phosphated Al/Fe-hydroxide co-precipitates with 0, 20, 50, 75, and 100 mol\% of total metal as Al (0-, 20-, 50-, 75-, 100-AFH) incubated with *S. putrefaciens* CN32 for 168 h: (a) 0-AFH, (b) 20-AFH, (c) 50-AFH, and (d) 75-AFH. Concentrations of sorbed PO$_4$ in the unit of mmol P mol$^{-1}$ Al+Fe are denoted by numbers in the legend. Spectra are rearranged to show the trend with PO$_4$ concentrations of (e) ~43 (46, 44, 44, 42, and 42 for 0-, 20-, 50-, 75-, and 100-AFH) and (f) ~130 (138, 132, 131, 126, and 125 for 0-, 20-, 50-, 75-, and 100-AFH) mmol mol$^{-1}$ Al+Fe across all samples.
Figure 4.9. Normalized P K-edge XANES spectra for 168-h-bioreduced 0-AFH with 138 mmol mol$^{-1}$ Al+Fe of sorbed PO$_4$ (0-AFH/P/bioreduction/168 h), PO$_4$ sorbed on 0- and 100-AFH at concentration of 181 mol$^{-1}$ Fe and of 166 mmol mol$^{-1}$ Al (0-, 100-AFH/P), crystalline and short-range order Fe(III)-PO$_4$ minerals [strengite, Fe(III)PO$_4$], and PO$_4$ sorbed on *S. putrefaciens* CN32 at concentration of 1000 mmol kg$^{-1}$ (CN32/P). The inset is the enlarged pre-white line region.
Figure 4.10. Phosphate distribution associated with Fe vs. Al on Al/Fe-hydroxide co-precipitates with 20, 50, and 75 mol% of total metal as Al (20-, 50-, 75-AFH) incubated with *S. putrefaciens* CN32 for 168 h determined by XANES analysis as the function of total sorbed PO₄. Dashed lines are reference lines (RL) that represent the expected Fe(III)-associated PO₄ if PO₄ was distributed with Fe(III) and Al(III) in proportion to their concentrations in the co-precipitates (non-preference). Linear regression fits to the data are shown as solid lines.
Figure A4.1. Phosphate sorption isotherms at pH 5.5 ± 0.05, 0.05 M KCl background electrolyte and the corresponding Freundlich models (solid lines) for Al/Fe-hydroxide co-precipitates with 0, 20, 50, 75, and 100 mol% of total metal as Al (0-, 20-, 50-, 75-, 100-AFH). Parameters $q_i$ and $c_i$ represent fitted sorbed and dissolved PO$_4$ concentrations on Freundlich model.
Figure A4.2. Normalized P K-edge XANES spectra for PO$_4$ sorbed at various concentrations (in mmol P mol$^{-1}$ Al+Fe shown as numbers in the legend) on Al/Fe-hydroxide co-precipitates with 20, 50, and 75 mol% of total metal as Al (20-, 50-, 75-AFH). See Table 4.1 for the exact sorption concentration.
Figure A4.3. Model fits (solid lines) to the Fourier transform of EXAFS spectra (open circles) for phosphated (92 mmol P mol\(^{-1}\) Al+Fe) Al/Fe-hydroxide co-precipitates containing 0 mol% of total metal as Al (0-AFH – Fe-hydroxide) incubated with \textit{S. putrefaciens} CN32 for 0.5 h. Scattering paths fitted using a structural model for two line-ferrihydrite [2-nm domain - (Michel et al., 2007)] illustrate the contribution from absorber atoms (Fe) to surrounding atoms (O and Fe).
CHAPTER 5
Conclusions and Implications

This research aimed to determine PO$_4$ retention and dissolution on assemblages of Al- and Fe-hydroxides as affected by the microbial-promoted reduction process. Corresponding molecular mechanisms were assessed using XAS techniques, including XANES and EXAFS spectroscopies. In the absence of Fe(III)-reducing bacteria, the majority of PO$_4$ was bound to surfaces of Al/Fe-hydroxide co-precipitates via adsorption mechanisms. Aluminum(III)-hydroxide polymers tended to locate on near-surfaces of co-precipitates and thereby provided greater accessibility for PO$_4$ retention, resulting in a preferential PO$_4$ bonding to Al in samples containing $\leq$ 50 mol% of Al. Meanwhile, such Al polymers may act to impede structural development in Fe-hydroxide domains. Along with the decreased structural development, the increased availability of Fe domains may offset the accessibility of Al polymers, leading a non-preferential PO$_4$ binding to either Al or Fe in samples containing 75 mol% of Al. The association of Al(III) and Fe(III) in poorly- or non-crystalline co-precipitates, as expected in nature, significantly changes the PO$_4$ adsorption properties relative to pure, isolated analogs. Our results indicate that differential precipitation of Al- vs. Fe-hydroxide domains affected both the adsorption capacity and the preferential bonding of PO$_4$.

The relative proportion of Al and Fe also has a substantial impact on the bioreducibility of Al/Fe-hydroxide co-precipitates. Reduction of Fe(III)-hydroxides is governed by geochemical and microbiological interactions, and thus, has been considered as
a complex process influenced by factors like aqueous chemistry, mineralogical characteristics, and activity of Fe(III)-reducers. In the absence of co-precipitated Al, the microbial-induced structural development in Fe(III)-hydroxide domains played a critical role limiting dissimilatory Fe(III) reduction. However, under identical incubation conditions, this structural transformation in Fe(III)-hydroxide domains was absent in co-precipitates containing 75 mol% of Al. The decreased structural ordering of Fe(III)-hydroxide domains would likely induce a more susceptible particle surface that can expedite electron transfer and further enhance the bioreducibility of Fe(III). Natural Fe-(hydr)oxides generally contain other cations such as Cr, Co, and Mn, and particularly Al. The ubiquity of Al in soils and sediments has generated interest in determining the effect of Al on dissimilatory Fe(III) reduction. Our results show evidence that Al, when present in co-precipitates, substantially enhanced Fe(III) bioreduction via optimizing the bioavailability of Fe domains. These impacts emphasize the need to consider the presence of Al while predicting the reducibility of dissimilatory Fe(III) reduction in aquatic and terrestrial environments.

Bioreduction of Al/Fe-hydroxide co-precipitates with sorbed PO$_4$ were also controlled by the relative structural ordering in Fe-hydroxide domains. In cases of Al content $\leq$ 20 mol%, the surface-sorbed PO$_4$ acted to retard the internal structural development in Fe-hydroxide domains, leading to a positive relationship between sorbed PO$_4$ concentration and the extent of Fe(III) reduction. However, sorbed PO$_4$ showed no effect in co-precipitates with Al $\geq$ 50 mol%, wherein the degree of interference in Fe-hydroxide polymerization caused by PO$_4$ effects was presumably less than that caused by Al co-precipitation. During bioreduction of phosphated Al/Fe-hydroxide co-precipitates, no PO$_4$ dissolution was detected.
Alternatively, Fe[(II)/(III)]-PO$_4$ bonding that increased with increasing extent of Fe bioreduction generally dominated solid-phase PO$_4$ in co-precipitates with $\leq 20$ mol% of Al. As Al proportion increased up to $\geq 50$ mol%, however, a preferential PO$_4$ bonding to Al mainly accounted for the PO$_4$ retention in residual solids.

Under the prerequisite that this study was conducted in model systems of Al/Fe-hydroxide co-precipitates, our findings do not support the common hypothesis that Fe(III)-associated PO$_4$ is more susceptible to dissolve under reducing conditions compared with Al(III)-associated PO$_4$. Instead, the association of labile PO$_4$ is proposed here to be controlled by the abundance of sorption sites on residual Al(III-) or Fe[(II)/(III)]-hydroxide limiting PO$_4$ dissolution. The reducibility of Fe(III) did not decrease the ability of residual Fe(III)-hydroxide domains to bind PO$_4$ under reducing conditions. However, competition with other ligands such as organic matter might decrease the availability of PO$_4$ binding sites in reduced soils. This study provided a molecular-scale insight to PO$_4$ fate as affected by changes in Al/Fe ratio in soil particles with high sorption capacity, and as affected by redox potential. My research improved the fundamental understanding needed to predict PO$_4$ mobility in natural complex environments such as flooded and wetland soils.