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3 **Effect of biofilm coatings at metal-oxide/water interfaces II:**
4 **Competitive sorption between Pb(II) and Zn(II) at *Shewanella***
5 ***oneidensis*/metal-oxide/water interfaces**

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Abstract:

Competitive sorption of Pb(II) and Zn(II) on *Shewanella oneidensis* MR-1 biofilm-coated single-crystal α -Al₂O₃ (1-102) and α -Fe₂O₃ (0001) surfaces was investigated using long-period x-ray standing wave-fluorescence yield (LP-XSW-FY) spectroscopy. *In situ* partitioning of aqueous Pb(II) and Zn(II) between the biofilm and underlying metal-oxide substrates was probed following exposure of these complex interfaces to equimolar Pb and Zn solutions (0.01M NaNO₃ as background electrolyte, pH = 6.0, and 3-hr equilibration time). At higher Pb and Zn concentrations ($\geq 10^{-5}$ M), more than 99% of these ions partitioned into the biofilms at *S. oneidensis*/ α -Al₂O₃ (1-102)/water interfaces, which is consistent with the partitioning behavior of both Pb(II) or Zn(II) in single-metal-ion experiments. Thus, no apparent competitive effects were found in this system at these relatively high metal-ion concentrations. However, at lower equi-molar concentrations ($\leq 10^{-6}$ M), Pb(II) and Zn(II) partitioning in the same system changed significantly compared to the single-metal-ion systems. The presence of Zn(II) decreased Pb(II) partitioning onto α -Al₂O₃(1-102) substantially (~52% to ~13% at 10^{-7} M, and ~23% to ~5% at 10^{-6} M), whereas the presence of Pb(II) caused more Zn(II) to partition onto α -Al₂O₃ (1-102) surfaces (~15% to ~28% at 10^{-7} M, and ~1% to ~7% at 10^{-6} M). The higher observed partitioning of Zn(II) (~28%) at the α -Al₂O₃ (1-102) surfaces compared to Pb(II) (~13%) in the mixed-metal-ion systems at the lowest concentration (10^{-7} M) suggests that Zn(II) is slightly favored over Pb(II) for sorption sites on α -Al₂O₃ (1-102) surfaces under our experimental conditions. Competitive sorption of Pb(II) and Zn(II) at *S. oneidensis*/ α -Fe₂O₃ (0001)/water interfaces at equi-molar metal-ion concentrations of $\leq 10^{-6}$ M showed that the presence of Pb(II) ions decreased Zn(II) partitioning onto α -Fe₂O₃(0001) significantly (~45% to <1% at 10^{-7} M, and ~41% to 3% at 10^{-6} M), whereas adding Zn(II) caused only small changes in Pb(II) partitioning (~59% to ~47% at 10^{-7} M, and ~26% to ~23% at 10^{-6} M), suggesting that Pb(II) strongly outcompetes Zn(II) for sorption sites on *S. oneidensis*-coated α -Fe₂O₃ (0001) surfaces. Our study implies that caution should be taken when applying results obtained from partitioning studies of single-metal-ion systems to mixed-metal-ion systems at complex biofilm/mineral interfaces.

1. INTRODUCTION

Although metal-ion sorption on Fe- and Al-(oxyhydr)oxides minerals has been well studied at the molecular level (e.g., Bargar et al., 1997a, 1997b; Brown and Sturchio, 2002), the mobility and bioavailability of metal ions in natural environments are still largely unknown due to the fact that mineral surfaces in soils can be extensively colonized by microbes or coated by natural organic matter (NOM) (Mayer, 1994; Barker et al., 1998; Ransom et al., 1999; Ganor et al., 2009; Bonneville et al., 2012; Lalonde et al., 2012). Minerals, microbial biofilms, and NOM have been shown to have strong affinities for metal ions and are good natural sorbents for heavy metal toxins (e.g., Fein et al., 1997; Brown et al., 1999; Tipping, 2002). Extensive studies have focused on metal-ion sorption on mineral surfaces (e.g., Brown and Sturchio, 2002), bacterial surfaces (e.g., Fein et al., 1997; Johnson et al., 2007), and/or soil NOM (e.g., Tipping, 2002). However, most of these studies investigated metal-ion sorption in single-substrate systems (e.g., mineral, bacteria, or NOM). As a result, metal cycling in more complex systems composed of a combination of microbial, organic, and mineral constituents remains under-explored and relatively poorly understood. This is due mainly to the inherent complexity of such systems and the lack of analytical tools capable of probing the partitioning of metal ions between different pools of reactive sites.

Recent advances in the use of high intensity synchrotron radiation, combined with the long period x-ray standing wave fluorescence yield (LP-XSW-FY) method, now enable studies of elemental distributions at a variety of buried interfaces and multi-layered substrates (Bedzyk et al., 1986; Bedzyk et al., 1988; Abruna et al., 1989; Bedzyk et al., 1990; Wang et al., 1991; Wang et al., 1992; Templeton et al., 2001; Templeton et al., 2003; Yoon et al., 2005; Kitts et al., 2009; Levard et al., 2011; Wang et al., submitted). The LP-XSW-FY technique uses the superposition of incoming and reflected x-ray beams on a reflecting surface under total external reflection conditions to generate a well-defined periodic electric field intensity variation known as an x-ray standing wave (XSW). Fluorescence yield of specific elements of interest is measured as a function of incident x-ray angle and quantified by XSW modelling to obtain the distribution and relative abundance of elements present both within a layer (film) and at an interface. The LP-XSW-FY technique has been reviewed in detail previously (Bedzyk and Cheng,

2002; Trainor et al., 2006) and several prior studies have demonstrated the utility of this approach for probing mineral substrates coated with biofilms/organic matter (Templeton et al., 2001; Templeton et al., 2003; Yoon et al., 2005; Wang et al., submitted). In the first LP-XSW-FY study of its kind, Templeton et al. (2001) evaluated the *in situ* partitioning of Pb(II) at *Burkholderia cepacia* biofilm-coated single-crystal metal-oxide surfaces. They found that the *B. cepacia* coating provided additional sorption sites and did not block reactive sites on the metal-oxide surfaces. We recently carried out LP-XSW-FY experiments on the partitioning of Pb(II) and Zn(II) at more environmentally realistic *Shewanella oneidensis* MR-1 biofilm/metal-oxide interfaces and obtained similar results (Wang et al., submitted), which suggests that these conclusions may be more general.

However, natural soil and aqueous systems have complex solution compositions; in many cases, a wide range of dissolved metals are present simultaneously and may interfere with each other for uptake and accumulation on mineral surfaces, in microbial biofilms, or in NOM (Benjamin and Leckie, 1981a; Fowle and Fein, 1999; Burnett et al., 2007). One can expect that various metal ions could compete to a greater or lesser extent for the available sorption sites. Therefore, studying competitive sorption effects in multi-component systems is essential for understanding metal-ion sorption in complex natural systems. Failure to account for competitive effects could lead to errors in predicting the fate and transport of the metal ions and, therefore, the toxicity to humans and biota in natural systems, as well as the efficiency of many engineered systems for heavy metal removal (Benjamin and Leckie, 1981a; Burnett et al., 2007).

A few studies to date have focused on competitive metal-ion sorption on bacterial surfaces (see, e.g., Mullen et al., 1989; Fowle and Fein, 1999; Burnett et al., 2007; Claessens and Van Cappellen, 2007). Mullen et al. (1989) investigated the adsorption of Ag(I), La(III), Cu(II), and Cd(II) by *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa* and found that metal-ion affinity to bacterial surfaces decreased in the order Ag(I)>La(III)>Cu(II)>Cd(II) (Mullen et al., 1989). Fowle and Fein (1999) studied mixed-metal-ion sorption of Cd(II), Cu(II), Pb(II), and Ca(II), by two Gram positive bacteria (*B. subtilis* and *B. licheniformis*) and found that the chemical equilibrium approach for single-metal-ion systems could be used to predict competitive sorption behavior in these multi-metal-ion systems (Fowle and Fein, 1999). Burnett et al.

(2007) performed multi-metal-ion competitive sorption experiments in which aqueous Cd(II), Cu(II), Mn(II), Ni(II), and Pb(II) ions were reacted simultaneously with the surfaces of the thermophile bacterium *Anoxybacillus flavithermus*, and found the following order of affinity for the bacterial surface: Mn(II)~Ni(II)<Zn(II)<Cd(II)<Pb(II)~Cu(II). They found that metal-ion stability constants obtained from single-metal-ion studies are comparable to those obtained from mix-metal-ion studies, although the stability constants obtained from single-metal-ion studies are more accurate. They also observed that high affinity metal ions outcompeted low affinity metal ions for sorption sites. Claessens and Van Cappellen (2007) studied Cu(II) and Zn(II) sorption on *Shewanella putrefaciens* planktonic cells and showed that Cu(II) binds more strongly to the cell walls than Zn(II), and that model parameters derived from single-metal-ion sorption could be used to account for the observed competitive sorption of Cu(II) and Zn(II) when both were present. However, these studies used planktonic cells, and to date no relevant competitive metal-ion sorption studies have been reported for systems containing complex biofilm/mineral interfaces.

Several studies have also considered competitive metal-ion sorption at mineral surfaces, and the effects have been found to range from none or weak to fairly strong competition, depending on the type of metal ions, type of surfaces, and experimental conditions (Gadde and Laitinen, 1974; Benjamin and Leckie, 1981a; Zhu et al., 2010). For example, the sorption of Cd(II) and Zn(II) on hydrous manganese oxide (HMO) at 10^{-4} M was reported to decrease significantly when in the presence of an equi-molar concentration of Pb(II) (Gadde and Laitinen, 1974). In contrast, Benjamin and Leckie (1981a) found no apparent competitive sorption among Cd(II), Cu(II), Zn(II), and Pb(II) on amorphous Fe(III)-oxyhydroxide. They hypothesized that discrete groups of sorption sites have different binding affinities for various metal ions that are present. Thus, the preferred sorption sites for Cd(II), Cu(II), Zn(II), and Pb(II) on amorphous Fe(III)-oxyhydroxides are thought to be distinct from each other, and no apparent competitive sorption was observed. Zhu et al. (2010) studied the competitive sorption of Cu(II), Cr(III), and Pb(II) on ferrihydrite and on two synthetic organo-mineral complexes formed by ferrihydrite and oxalate or tartrate. They found that competitive sorption of metal ions in their systems depends on a number of factors including the type of metal ions, the

initial metal-ion concentrations used, the surface properties of the sorbents, the sequence of metal ions added, and reaction time.

Despite various studies of competitive metal-ion sorption on bacterial and mineral surfaces, to the best of our knowledge no studies of multi-metal-ion competitive sorption in biofilm-coated mineral systems have been published to date. Prior work on single metal-ion systems has shown that a microbial biofilm coating or natural organic matter coating does not block available binding sites on metal-oxide surfaces for the systems studied (Templeton et al., 2001; Yoon et al., 2005; Wang et al., submitted); however, it is not known whether competition between different metal ions is a significant factor in metal-ion partitioning between the microbial biofilm/organic coatings and metal-oxide substrates. One advantage of using the LP-XSW-FY technique is that the incident x-ray beam energy can be tuned in energy to simultaneously excite multiple elements present in the system, which allows the study of competitive sorption effects among various metal ions both within the biofilms and at the mineral surfaces. In this study, we applied the LP-XSW-FY technique to probe the *in situ* partitioning of Pb(II) and Zn(II) present simultaneously at equi-molar concentrations in systems composed of *S. oneidensis*-coated single-crystal α -Al₂O₃ (1-102) and α -Fe₂O₃ (0001) surfaces. The work presented here focuses on competitive sorption between Pb(II) and Zn(II), therefore, it is a companion to Part I (also in this volume) which was concerned with single Pb(II) and Zn(II) partitioning.

2. MATERIALS AND METHODS

2.1 Sample preparation

Commercially available, highly polished α -Al₂O₃ (1-102) single crystals (Saint-Gobain Crystals & Detectors Co.) and natural hematite α -Fe₂O₃ (0001) (variety specularite) single crystals from Bahia, Brazil, were used as the metal-oxide substrates in this study. The preparation, cleaning, and characterization procedure for these crystals and the protocol to grow *S. oneidensis* MR-1 biofilms are described in Part I (Wang et al., submitted). *S. oneidensis* MR-1 biofilms were grown for 10 days aerobically at pH 7.0 on the single-crystal substrates in flow-through reactors in a 30°C constant temperature room using the nutrient medium defined in Part I (Wang et al., submitted).

Sodium pyruvate (0.5 mM) was used as a carbon and electron source. All media were freshly prepared and autoclaved and pumped into the flow-through reactors using a peristaltic Watson/Marlow pump (model 205S/CA, Wilmington, MA). After a 10-day growth period and a flushing procedure using minimum medium, the biofilm-coated substrates were carefully removed from each reactor under sterile conditions and rinsed three consecutive times in fresh sterile 0.01 M NaNO₃ solution.

Reagent grade Pb(NO₃)₂ and Zn(NO₃)₂ (J.T. Baker) were used to prepare metal stock solutions. Solutions containing equi-molar concentrations of Pb(II) and Zn(II) ranging from 10⁻⁴ M to 10⁻⁷ M were prepared using N₂-sparged 0.01 M NaNO₃ aqueous solution as the background electrolyte, and the pH was adjusted to 6.00 ± 0.05. Rinsed biofilm-coated single-crystal samples were gently immersed in 35-ml Pb(II)- and Zn(II)-containing aqueous solution of a specific concentration. The solution pH was checked frequently during metal exposure experiments and any deviation from pH 6.0 was adjusted by adding small volumes of NaOH or HNO₃ solutions (0.001 to 0.01 M). The biofilm-coated metal-oxide samples were carefully withdrawn from the solution after reaction with Pb(II) and Zn(II) containing aqueous solutions for 3 hours, then mounted on an enclosed sample holder for LP-XSW-FY measurements, and humidified helium gas was flowed through the sample holder to keep the sample under hydrous conditions.

2.2 LP-XSW-FY measurements and data analysis

LP-XSW-FY measurements were performed at GeoSoilEnviroCARS (GSECARS) on insertion device beamline 13-ID-C at the Advanced Photon Source (APS) using a general-purpose diffractometer in horizontal scattering geometry. The x-ray optics configuration and sample mounting procedure are described in Part 1 (Wang et al., submitted). In brief, a 14 keV highly collimated (divergence < 0.001°) x-ray beam was scanned between incidence angles of 0.0 and 0.5° for x-ray reflectivity measurements by monitoring the incident (I₀) and reflected (I₁) x-rays through N₂-filled gas ionization chambers. Fluorescence yield data were simultaneously collected at 14 keV incident x-ray energy using a 4-element silicon drift detector (SII NanoTechnology, Vortex-ME4) coupled to digital x-ray processor electronics (X-ray Instrumentation Associates, xMAP). During LP-XSW-FY data collection, the initial alignment was performed at one position

for a sample, and each subsequent LP-XSW-FY profile was then collected at different spots after quick realignment to reduce beam exposure. The sample was moved perpendicular to its surface normal and transversely relative to the beam direction by at least 1 mm from the initial alignment position with a minimum of two replicate FY profiles collected for each sample.

LP-XSW-FY data analysis includes three major steps: (1) extracting the x-ray fluorescence intensity of each element from a full x-ray fluorescence spectrum measured with an energy dispersive detector; (2) fitting the specular x-ray reflectivity to calculate the LP-XSW-FY field intensity modulation; and (3) modeling the distribution function of the fluorescing species and combining the distribution function with the electric field intensity modulation (Templeton et al., 2001; Yoon et al., 2005; Kitts et al., 2009; Levard et al., 2011; Wang et al., submitted). A two-box model was used which consisted of two regions: one near the substrate surface with a thickness of 5 Å and another extending from 5 Å to the top of the biofilms. The total FY of the sample, which includes contributions from each box of the model, is roughly proportional to the total number of fluorescing atoms. Finally, a least-square fitting routine was used to fit the parameters of the distribution model from which the element partitioning profiles were obtained. The fitting was performed using custom-made code (Kitts et al., 2009) in the Igor program (Igor Pro, WaveMetrics, Lake Oswego, Oregon). More detailed information about modeling and data analysis can be found in Part I (Wang et al., submitted).

3. RESULTS

3.1 Pb(II) and Zn(II) partitioning at *S. oneidensis*/α-Al₂O₃ (1-102)/water interfaces

Figs. 1a and 1b show the x-ray reflectivity and LP-XSW-FY profiles for Pb(II) and Zn(II) partitioning at equi-molar concentrations ranging from 10⁻⁴ to 10⁻⁷ M at *S. oneidensis* MR-1 biofilm-coated α-Al₂O₃ (1-102) surfaces after 3 hr of equilibration time. Consistent with our previous studies (Templeton et al., 2001; Yoon et al., 2005; Wang et al., 2013; Wang et al., submitted), the critical angle of the α-Al₂O₃ (1-102) surface was found to be 0.165° ± 0.010° at an incidence x-ray energy of 14 keV. The reflectivity profiles do not show fine structure because of the low density and high surface roughness of the biofilms. For Pb(II) partitioning at concentrations ranging from

10⁻⁴ to 10⁻⁵ M, the LP-XSW-FY profiles show two broad overlapping peaks with maximum intensity in the low angle region at ~0.1°, suggesting that the biofilms dominate the sorption of Pb(II) under these conditions (Fig. 1a). At the lower concentrations (10⁻⁶ to 10⁻⁷ M), the relative distribution of the FY intensity from Pb systematically changes, with the FY intensity increasing in the peak centered at ~0.16°. The increased FY intensity in this region, which is closer to the critical angle of α -Al₂O₃ (1-102) surface, indicates that Pb(II) increasingly partitioned onto the α -Al₂O₃ (1-102) surfaces as Pb concentration decreased (Fig. 1a).

The Zn(II) LP-XSW-FY profiles (Fig. 1b) show features similar to those for Pb(II) as a function of Zn concentration. At higher concentrations ($\geq 10^{-5}$ M), Zn(II) FY profiles exhibit the maximum intensity in the lower angle region ~0.1°, suggesting that the biofilms dominated the sorption of Zn(II) (Fig. 1b). As the concentration decreased, similar to the case of Pb(II), we observed an increase in the relative Zn(II) FY intensities at ~0.16°, indicating that Zn(II) increasingly partitioned onto the α -Al₂O₃ (1-102) surfaces with decreasing Zn concentration. At the lowest concentration tested (10⁻⁷ M), the Zn(II) FY profile has a broad maximum close to the critical angle of α -Al₂O₃(1-102) that is slightly more intense than the broad maximum at ~0.08°. In contrast, the intensity of the broad maximum at ~0.16° in the Pb(II) FY profile at 10⁻⁷ M Pb is lower than the maximum at ~0.08°, indicating that the partitioning of Zn(II) onto the α -Al₂O₃ (1-102) surface is higher than that of Pb(II) at Pb and Zn concentrations of 10⁻⁷ M.

Quantitative information on Pb(II) and Zn(II) partitioning at equi-molar concentrations between *S. oneidensis* MR-1 biofilms and α -Al₂O₃ (1-102) surfaces can be obtained by LP-XSW-FY profile fitting using the two-box model described in section 2.2. As shown in Table 1, at an initial Pb(II) concentration of 10⁻⁷ M, ~13% of Pb(II) partitioned onto the α -Al₂O₃ (1-102) surface compared to ~87% into the biofilms, whereas ~28% of Zn(II) partitioned onto the α -Al₂O₃ (1-102) surfaces compared to ~72% into the biofilms. At Pb(II) and Zn(II) concentration of 10⁻⁶ M, ~5% of Pb(II) partitioned onto the α -Al₂O₃ (1-102) surface compared to ~95% into the biofilms, whereas ~7% of Zn(II) partitioned onto the α -Al₂O₃ (1-102) surfaces compared to ~93% into the biofilms. At $\geq 10^{-5}$ M, both Pb(II) and Zn(II) preferentially partitioned into the biofilms (>99%),

which indicates that the biofilms became the dominant sinks for both metal ions and no apparent competitive sorption was observed at these higher concentrations.

Figs. 2a and 2b illustrate the effects of competition between Pb(II) and Zn(II) ions on *S. oneidensis* MR-1 biofilm-coated α -Al₂O₃ (1-102) surfaces compared to single-metal-ion systems. In comparison to Pb(II) partitioning in the single-ion system, adding an equi-molar concentration of Zn(II) decreased the amount of Pb(II) partitioning onto α -Al₂O₃ (1-102) surfaces significantly after 3 hr of exposure at low metal-ion concentrations (10^{-6} to 10^{-7} M) (Fig. 2a). In Part I, when only Pb(II) was present at 10^{-7} M, we observed preferential partitioning of Pb(II) onto α -Al₂O₃ (1-102) surfaces (Wang et al., submitted). However, when an equi-molar concentration (10^{-7} M) of Zn(II) was present in the system, Pb(II) partitioning decreased from ~52% (Wang et al., submitted) to ~13% (Table 1), and the α -Al₂O₃ (1-102) surfaces were no longer the dominant sinks for Pb(II). Similar competitive effects were noted at metal-ion concentrations of 10^{-6} M, where adding an equi-molar concentration of Zn(II) to the system decreased Pb(II) partitioning onto the α -Al₂O₃ (1-102) surface from ~23% (Wang et al., submitted) to ~5% (Table 1). At higher metal-ion concentrations ($\geq 10^{-5}$ M), the biofilms dominated the sorption of Pb(II) in both single-metal-ion (Wang et al., submitted) and mixed-metal-ion systems (>99%), and thus no apparent competitive effects were observed (Table 1).

Compared with the effect of Zn(II) on Pb(II) described above, adding an equi-molar concentration of Pb(II) to the Zn(II)/*S. oneidensis*/ α -Al₂O₃ (1-102) system increased Zn(II) partitioning onto α -Al₂O₃ (1-102) surfaces at lower concentrations ($\leq 10^{-6}$ M). As shown in Fig. 2b and Table 1, adding an equi-molar concentration of Pb(II) caused a small increase in the partitioning of Zn(II) to the α -Al₂O₃ (1-102) surface from ~15% (Wang et al., submitted) to ~28% and from <1% (Wang et al., submitted) to 7% at the lower concentrations of 10^{-7} M and 10^{-6} M, respectively. At higher concentrations ($\geq 10^{-5}$ M), Zn(II) partitioning in both single-metal-ion (Wang et al., submitted) and mixed-metal-ion systems was dominated by the biofilms (>99% in the biofilms), and thus no apparent competitive effects were observed (Fig. 2b and Table 1).

In comparison with the partitioning behavior of Zn(II) and Pb(II) in single-metal-ion systems at low concentrations ($\leq 10^{-6}$ M), adding Zn(II) and Pb(II) simultaneously at equi-molar concentrations resulted in one component, Pb(II), partitioning more into the

biofilms and less onto the α -Al₂O₃ (1-102) surfaces, whereas the same conditions caused the second component, Zn(II), to partition more to the α -Al₂O₃ (1-102) surfaces and less into the biofilms. The partitioning behavior of Zn(II) and Pb(II) in the mixed-metal-ion systems at low metal-ion concentrations suggests competition for available binding sites both at the metal-oxide surfaces and in the *S. oneidensis* MR-1 biofilms.

3.2 Pb(II) and Zn(II) partitioning at *S. oneidensis*/ α -Fe₂O₃ (0001)/water interfaces

Pb(II) and Zn(II) partitioning on *S. oneidensis* MR-1 biofilm-coated α -Fe₂O₃ (0001) surfaces was also evaluated as a function of equi-molar metal-ion concentrations using LP-XSW-FY spectroscopy. Figs. 3a and 3b show the x-ray reflectivity and LP-XSW-FY profiles for Pb(II) and Zn(II) partitioning at equi-molar concentrations ranging from 10⁻⁵ to 10⁻⁷ M at *S. oneidensis* MR-1 biofilm-coated α -Fe₂O₃ (0001) interfaces after 3 hr of equilibration time, which consist of two broad and overlapping maxima. Due to the higher electron density than α -Al₂O₃, the critical angle of the α -Fe₂O₃ (0001) surface is larger than α -Al₂O₃ and was found to be 0.185° ± 0.010° at an incident x-ray energy of 14 keV. Again, the low-angle peak is centered at ~0.1° and corresponds to the presence of Pb(II) or Zn(II) in the *S. oneidensis* MR-1 biofilm. The second peak corresponding to the presence of Pb(II) or Zn(II) at the α -Fe₂O₃ substrate is centered at ~0.18°, close to the measured critical angle of α -Fe₂O₃. At low initial metal-ion concentration (10⁻⁷ M), the Pb(II) LP-XSW-FY profile showed an intensity maximum close to the α -Fe₂O₃ critical angle, suggesting that a significant amount of Pb(II) ions sorbed on the α -Fe₂O₃ (0001) surface. Increasing the initial Pb(II) concentration to 10⁻⁶ M resulted in two FY maxima with approximately equal intensities. The increase in intensity at lower angles (~0.1°) suggests that Pb(II) partitioned more into the biofilms with increasing metal-ion concentration. At the highest initial Pb(II) concentration (10⁻⁵ M), the low-angle maximum (~0.1°) dominates, indicating that the biofilm became the dominant sink for Pb(II) sorption. In contrast, the LP-XSW-FY profiles of Zn(II) partitioning at the biofilm-coated α -Fe₂O₃ (0001) surface (Fig. 3b) consistently showed the highest intensity in the low angle region (~0.1°) over the concentration range evaluated, suggesting that the biofilms are the dominant sinks for Zn(II) at all three concentrations tested.

Table 1 presents the percentage of Pb(II) and Zn(II) partitioning at both the biofilms and surfaces obtained from LP-XSW-FY profile fitting using the two-box model described in Section 2.2. At the highest concentration (10^{-5} M) studied for this system, both Pb(II) and Zn(II) LP-XSW-FY profiles show maxima at $\sim 0.1^\circ$, which suggests that biofilms dominated the sorption of both Pb(II) and Zn(II) (see Figs. 3a and 3b). In addition, LP-XSW-FY fitting results (Table 1) show that more than 99% of Pb(II) and Zn(II) sorbed into the biofilms. At initial equi-molar Pb(II) and Zn(II) concentrations of 10^{-6} M, significant increases in the FY signals close to the critical angle of $\alpha\text{-Fe}_2\text{O}_3$ (0001) surfaces were observed for Pb(II) relative to Zn(II): $\sim 23\%$ of Pb(II) and $\sim 3\%$ of Zn(II) partitioning onto the $\alpha\text{-Fe}_2\text{O}_3$ (0001) surfaces. However, at the lowest concentration (10^{-7} M), the FY maximum for Pb(II) was close to the critical angle of $\alpha\text{-Fe}_2\text{O}_3$ (0001) surfaces (see Fig. 3a), indicating that more Pb(II) ions partitioned onto $\alpha\text{-Fe}_2\text{O}_3$ (0001) surfaces ($\sim 47\%$ - Table 1). In contrast, the FY maximum of Zn(II) was close to $\sim 0.1^\circ$ (see Fig. 3b), indicating that Zn(II) ions partitioned mainly into the biofilms ($>99\%$ as shown in Table 1).

Figs. 4a and 4b illustrate the effects of adding equi-molar Pb(II) and Zn(II) concentrations to the *S. oneidensis* MR-1 biofilm-coated $\alpha\text{-Fe}_2\text{O}_3$ (0001) surfaces compared to the single-metal-ion systems. In comparison to single-metal-ion Pb(II) partitioning under the same conditions, adding Zn(II) and Pb(II) at equi-molar concentrations to the system slightly decreased the amount of Pb(II) partitioning onto $\alpha\text{-Fe}_2\text{O}_3$ (0001) surfaces. For example, $\sim 59\%$ of the Pb(II) ions partitioned to the $\alpha\text{-Fe}_2\text{O}_3$ (0001) surface (Wang et al., submitted) when only Pb(II) was present at 10^{-7} M compared to $\sim 47\%$ when both Zn(II) and Pb(II) were present at equi-molar concentrations (Table 1). At higher concentrations of 10^{-5} and 10^{-6} M, Pb(II) partitioning onto $\alpha\text{-Fe}_2\text{O}_3$ (0001) surfaces was comparable in both the single- and the mixed-metal-ion systems, suggesting no strong competitive sorption effects of Zn(II) on Pb(II) under these conditions. In contrast, the effect of Pb(II) on the partitioning behavior of Zn(II) was substantial. Pb(II) significantly reduced Zn(II) sorption onto $\alpha\text{-Fe}_2\text{O}_3$ (0001) surfaces at lower concentrations of 10^{-6} M and 10^{-7} M (Fig. 4b). For example, Zn(II) partitioning onto $\alpha\text{-Fe}_2\text{O}_3$ (0001) surfaces decreased from $\sim 45\%$ (Wang et al., submitted) to $\sim 1\%$ at 10^{-7} M,

and from ~41% (Wang et al., submitted) to ~3% at 10^{-6} M, respectively, in the presence of equi-molar Pb(II) (Table 1).

4. DISCUSSION

4.1 Competitive effects between Pb(II) and Zn(II) at *S. oneidensis*/ α -Al₂O₃ (1-102)/water interfaces

The effect of Zn(II) decreasing the amount of Pb(II) partitioning onto the α -Al₂O₃ (1-102) surface when both Pb(II) and Zn(II) were present at equi-molar concentrations suggests that Zn(II) outcompetes Pb(II) for the reactive α -Al₂O₃ (1-102) surface sites (see Section 3.1). Differences in binding affinities of Pb(II) and Zn(II) to the α -Al₂O₃ (1-102) surface must be considered in order to rationalize the observed trend. Table 2 lists the available site densities and binding affinities of Pb(II) and Zn(II) for several bacterial surfaces and metal oxides. Unfortunately, there are no log *K* data available for the binding of Zn(II) on α -Al₂O₃ (1-102) surfaces, so a thorough analysis for Zn(II) sorption in these complex mixed-metal-ion systems is not possible. However, some past studies have shown that Zn(II) prefers alumina surfaces more than Pb(II) (Davis and Leckie, 1978; Coston et al., 1995). For example, Coston et al. (1995) studied Pb(II) and Zn(II) sorption on natural Al- and Fe-oxide surface coating on an aquifer sand and found that removing the aluminum oxide coatings significantly reduced Zn(II) sorption compared to Pb(II) sorption. This finding suggests that Zn(II) preferred to form complexes with reactive sites on the aluminum oxide coatings more than Pb(II) (Coston et al., 1995). Other studies of Pb(II) and Zn(II) adsorption by individual metal oxides suggest a strong preference of Pb(II) for Fe-oxides in comparison with Al-oxides, whereas Zn(II) adsorption is similar for both oxides (Davis and Leckie, 1978; McKenzie, 1980; Benjamin and Leckie, 1981b). As shown in Table 2, the binding affinity of Pb(II) to γ -Al₂O₃ was found to be lower than or comparable to that of Zn(II) for monohydroxyl monodentate complexes depending on the concentration of Pb(II) and Zn(II) (Davis and Leckie, 1978). This result suggests that Zn(II) ions may have a higher affinity for the α -Al₂O₃ (1-102) surface relative to Pb(II) ions. Therefore, stronger partitioning of Zn(II) onto the α -Al₂O₃ (1-102) surfaces is expected when Pb(II) is present on the surface. We note that this effect is also consistent with our LP-XSW-FY study on competitive sorption of Pb(II)

and Zn(II) on polyacrylic acid (PAA)-coated α -Al₂O₃ (1-102) surfaces, where Zn(II) outcompeted Pb(II) on the α -Al₂O₃ (1-102) surface when both Pb(II) and Zn(II) were present at equi-molar concentrations (Wang et al., 2013).

Based on the above results, the competition between the two ions for α -Al₂O₃ (1-102) surface sites may explain the observed increase in Zn(II) partitioning and decrease in Pb(II) partitioning at α -Al₂O₃ (1-102) surfaces when both metal ions are present. An alternative explanation for this observation is that Pb(II) outcompetes Zn(II) for the sorption sites in the *S. oneidensis* MR-1 biofilms and indirectly causes the amount of Zn(II) sorption on the metal-oxide surface to increase. Several studies of Pb(II) and Zn(II) sorption on bacterial surfaces have shown that the affinity constants of Pb(II) ions are generally higher than those of Zn(II) ions as shown in Table 2 (Loaec et al., 1997; Ha et al., 2010). Loaec et al. (1997) studied the competition between Pb(II) and Zn(II) in uptake experiments on the exopolysaccharide (EPS) isolated from *Alteromonas macleodii* subsp *fijiensis* and found that Pb(II) was preferentially complexed by EPS. The binding affinities of Pb(II) and Zn(II) to EPS were found to be ~5.2 and ~4.4, respectively. Ngwenya et al. (2003) determined the binding affinities of Pb(II) and Zn(II) to carboxyl groups to be 3.9 (± 0.8), and 3.3 (± 0.1), respectively. The average binding affinities of Pb(II) to carboxyl groups and phosphoryl groups for *S. oneidensis* MR-1 planktonic cells were found to be 4.6 and 4.1, respectively, whereas the binding affinities of Zn(II) to carboxyl groups and phosphoryl groups were found to be 3.6 and 3.7, respectively, by Ha et al. (2010). Thus, it is expected that Pb(II) should outcompete Zn(II) for reactive binding sites in *S. oneidensis* MR-1 biofilms, resulting in a higher effective concentration of free Zn(II) ions at the Al₂O₃ (1-102) surfaces and increased sorption of Zn(II) on these surfaces.

However, caution should be taken when using the binding affinities of Pb(II) and Zn(II) reported in Table 2 because they were obtained under experimental conditions that may be significantly different from those used in the present study. For example, the available binding affinities for Zn(II) listed in Table 2 are for γ -Al₂O₃ powders and not for α -Al₂O₃ (1-102) single-crystal surfaces. These binding affinities were also found to vary as a function of metal-ion concentrations and types of surface complexes formed such as AlO-Me²⁺ or AlO-MeOH⁺ (Davis and Leckie, 1978). The log *K* of Pb(II) sorption

on α -Al₂O₃ (1-102) single-crystal surfaces (6.0) reported by Templeton et al. (2001) is close to that reported for AlO-Pb²⁺ monodentate complexes on γ -Al₂O₃, but it is about 3-4 log units lower than the log *K* (10.2) reported for AlO-PbOH⁺ monohydroxyl complexes on γ -Al₂O₃ (Davis and Leckie, 1978). In addition, the results of grazing-incidence x-ray absorption fine structure (GI-XAFS) spectroscopy studies of Zn(II) and Pb(II) ion sorption at α -Al₂O₃ (1-102) surfaces have shown that both metal ions form dominantly mononuclear edge-sharing bidentate complexes (Bargar et al., 1996; Trainor et al., 2001). It is expected that the log *K* value of bidentate complexes should be higher than that of the monodentate complexes reported in Table 2 for Pb(II) and Zn(II). On the other hand, the log *K* values of Pb(II) and Zn(II) in Table 2 were obtained from planktonic *S. oneidensis* MR-1 cells, and it is not known how they differ from the log *K* values of Pb(II) and Zn(II) in the biofilm matrix. Therefore, it is difficult to make any direct comparison of these reported log *K* values. New thermodynamic data for metal/mineral and metal/biofilm interactions are required to rationalize the observed competitive effects between Pb(II) and Zn(II) at complex *S. oneidensis* MR-1 biofilm/ α -Al₂O₃ (1-102)/water interfaces.

As the initial Pb(II) and Zn(II) concentrations increase in the *S. oneidensis* MR-1 biofilm/ α -Al₂O₃ (1-102)/water system, both Pb(II) and Zn(II) increasingly partition into the biofilms. This is consistent with the trends of Pb(II) and Zn(II) partitioning as a function of metal-ion concentration observed in the single-metal-ion systems examined in Part I (Wang et al., submitted). Based on the Langmuir model analysis described in Part I, at low metal concentrations the surface sites of α -Al₂O₃ (1-102) are more energetic than the various types of functional groups in the *S. oneidensis* biofilm and become the preferential sorption sites for metal ions. Unlike the single-metal-ion partitioning results, no preferential partitioning on α -Al₂O₃ (1-102) surfaces was observed for either Pb(II) or Zn(II) even at the lowest concentration tested when both metal ions were present at equimolar concentrations. As the strongly reactive metal-oxide surface sites become increasingly occupied, the metal ions partition more to the weaker biofilm sorption sites. At higher concentrations ($\geq 10^{-5}$ M), the biofilms are the dominant sinks for metal ions compared to α -Al₂O₃ (1-102) surfaces. Because modeling of the LP-XSW-FY profiles is of limited sensitivity in this concentration region as discussed in Wang et al. (submitted)

(>99% in the biofilms at concentrations $\geq 10^{-5}$ M), only semi-quantitative distributions can be obtained. Therefore, no apparent competitive effects between Pb(II) and Zn(II) were observed at higher Pb(II) and Zn(II) concentrations (10^{-4} M and 10^{-5} M).

4.2 Competitive effects between Pb(II) and Zn(II) at *S. oneidensis*/α-Fe₂O₃ (0001)/water interfaces

The difference in Pb(II) and Zn(II) partitioning at lower equi-molar concentrations in single-metal-ion and mixed-metal-ion systems suggests that Pb(II) exhibits a significant competitive sorption effect on Zn(II), in particular, in terms of outcompeting Zn(II) ions for reactive binding sites on the α-Fe₂O₃ (0001) surface (see Section 3.2). This trend is similar to that observed for the uncoated metal-oxide surfaces by Coston et al. (1995), where Pb(II) sorption was found to be significantly reduced compared with Zn(II) after removing iron-oxide coatings, suggesting that Pb(II) preferred to form complexes with reactive sites on iron-oxides (Coston et al., 1995). Some studies of Pb(II) and Zn(II) adsorption on metal oxides suggest a strong preference of Pb(II) for adsorption by Fe(III)-oxides compared to Al oxides (Davis and Leckie, 1978; McKenzie, 1980; Benjamin and Leckie, 1981b).

Differences in the binding affinities of Pb(II) and Zn(II) to the α-Fe₂O₃ (0001) surface are needed to help explain the observed competition between Pb(II) and Zn(II) at *S. oneidensis* MR-1 biofilm/α-Fe₂O₃ (0001)/water interfaces at low metal-ion concentrations. As shown in Table 2, the log *K* of Pb(II) on α-Fe₂O₃ (0001) surfaces was found to be 6.65 by Templeton et al. (2001). Unfortunately, there are no data available for log *K* values of Zn(II) on α-Fe₂O₃ (0001) surfaces. Davis and Leckie (1978) showed that the log *K* of monohydroxyl Pb(II) complexes is 1.7 units higher than that of Zn(II) on amorphous Fe(III)-oxides. Therefore, Pb(II) is expected to compete with Zn(II) for sorption sites on α-Fe₂O₃ (0001) surfaces. However, the log *K* value (11.1) for FeO⁻-PbOH⁺ monohydroxyl complexes reported by Davis and Leckie (1978) is much higher than that (6.65) reported by Templeton et al. (2001) for Pb(II) on single-crystal α-Fe₂O₃ (0001) surfaces, although the log *K* value (6.9) for monodentate Pb(II) complexes is closer to what Templeton et al. (2001) found. The differences are also significant between the log *K* values reported for Pb(II) and Zn(II) on iron-oxide surfaces (6.65-

11.1) and between the log K values reported for bacterial surfaces (3.6-4.6). If these strong sorption sites are present on the α -Fe₂O₃ (0001) surfaces, then they should dominate the sorption of Pb(II) and Zn(II) versus the functional groups in *S. oneidensis* MR-1 biofilms at low metal-ion concentrations. The result of this analysis is a further indication that the intrinsic differences in reactivity of the metal-oxide substrates with respect to aqueous Pb(II) and Zn(II) are preserved when coated by multi-layer *S. oneidensis* MR-1 biofilms.

4.3 Kinetic aspects of Pb(II) and Zn(II) sorption at *S. oneidensis*/metal oxide/water interfaces

Because of the complex nature of the biofilm structure, the competitive effects observed between Pb(II) and Zn(II) at *S. oneidensis* MR-1 biofilm/metal-oxide/water interfaces may result from kinetically controlled processes. This is thought to be the case because Pb(II) and Zn(II) ions must diffuse through the biofilm coatings to reach the underlying metal-oxide surfaces. The rate at which metal ions diffuse through the biofilms is expected to play an important role in metal partitioning at such complex interfaces. It is known that metal-ion sorption kinetics often are fast initially followed by a slow reaction (Covelo et al., 2004). The fast reaction step is likely due to the formation of inner-sphere complexes of metal ions with biofilms and mineral surfaces, as well as weaker binding of metal ions through electrostatic interactions. The slow sorption step may involve processes such as slow diffusion of metal ions through the biofilms to mineral surfaces, formation of surface precipitates, and metal-ion adsorption onto sites that have relatively large activation energies (Covelo et al., 2004).

The diffusion of free Pb(II) and Zn(II) ions in solution should also be considered in the biofilm systems because of the presence of water channels in the biofilm structure (Stoodley et al., 1994). Table 3 lists the ionic radii, hydrated radii, water exchange rates, and diffusion coefficients of Pb(II) and Zn(II) reported in the literature. Pb(II) ions have a larger ionic radius than Zn(II) ions; however, the hydrated radius of Pb(II) ions is smaller than that of Zn(II) ions, as shown in Table 3. Therefore, free Pb(II) ions should have a larger diffusion coefficient than free Zn(II) ions in dilute simple electrolytes such as KCl or NaNO₃ (Wang, 1954). In contrast, Pb(II) ions have a faster water exchange rate than

Zn(II) ions by almost two orders of magnitude (Stumm and Morgan, 1996). These differences lead to the prediction of faster diffusion of free Pb(II) ions through the biofilm coatings and sorption onto the metal-oxide surfaces than free Zn(II) ions.

However, metal ions are expected to interact with carboxyl and phosphoryl functional groups in the biofilms, and form metal-organic complexes. The stronger the metal-organic complexes, the more difficult it is for the metal ions to dissociate from the metal-organic complexes and diffuse to mineral surfaces. It is noted that the diffusion coefficients are quite small for metal-organic complexes such as metal-ion-polyacrylic acid (PAA) complexes (PAA is a simplified model for carboxyl functional groups in NOM) in comparison to metal ions in simple electrolytes, as shown in Table 3. It was found that the stronger the metal ions bind to organics such as PAA, the smaller the diffusion coefficient of metal ions in media when Na-PAA was used as the electrolyte (Kojima and Fujiwara, 1970). For example, the diffusion coefficient of CuCl_2 was found to be $6 \pm 3 \times 10^{-11} \text{ cm}^2/\text{sec}$, whereas that of ZnCl_2 was found to be $2.4 \pm 0.3 \times 10^{-9} \text{ cm}^2/\text{sec}$ when Na-PAA was used as the electrolyte (Kojima and Fujiwara, 1970). No diffusion coefficient for Pb(II) in PAA was reported in that study. Considering that Pb(II)-PAA and Cu(II)-PAA have similar stability constants, as shown in several studies (Morlay et al., 1998; Roma-Luciw et al., 2001; Tomida et al., 2001), the diffusion coefficient of Pb(II) in PAA should be similar to that of Cu(II) in PAA. Thus it is likely that Pb(II) diffusion in PAA is about two orders of magnitude slower than Zn(II) diffusion in PAA. Using PAA as a model compound for carboxyl functional groups in the biofilms, it is expected that the diffusion of Pb(II) in the biofilms should be much slower than that of Zn(II).

In summary, free Pb(II) ions should have faster diffusion kinetics than free Zn(II) ions; however, stronger Pb(II) binding to functional groups in the biofilm should result in slower diffusion kinetics than more weakly bound Zn(II). Thus, it is very difficult to predict the overall kinetics of metal-ion sorption in these complex systems. As discussed in Part I (Wang et al., submitted), both Pb(II) and Zn(II) ions partitioned more onto underlying metal-oxide substrates at longer reaction time (20-24 hrs) for single-metal-ion systems. For mixed-metal-ion systems, we expect both Pb(II) and Zn(II) ions to partition more onto metal-oxide surfaces at longer reaction times. However, at 3-hr reaction time, the competitive effects between Pb(II) and Zn(II) at both metal-oxide surfaces observed

in this study are consistent with the available thermodynamic data (see section 4.1 and 4.2), indicating that such competitive effects between Pb(II) and Zn(II) in the systems we studied are thermodynamically controlled. Therefore, we predict that similar competitive effects between Pb(II) and Zn(II) would be observed for longer reaction times. Future studies that focus on both kinetics and thermodynamics are needed to better understand metal-ion partitioning at complex biofilm/mineral/water interfaces.

5. CONCLUSIONS

Competitive sorption effects between Pb(II) and Zn(II) on *S. oneidensis* MR-1 biofilm-coated α -Al₂O₃ (1-102) and α -Fe₂O₃ (0001) surfaces were studied using LP-XSW-FY spectroscopy. When both ions were present, biofilms became the dominant sinks for both Pb(II) and Zn(II) in the systems after 3 hr of reaction time, even at the lowest metal concentration tested (10^{-7} M) on both surfaces. This result is consistent with the idea that reactive sorption sites on the surfaces are more filled when both metal ions are present. The amount of Pb(II) and Zn(II) partitioning into the biofilms increased as their concentrations increased, which is consistent with the trend found in the single-metal-ion systems. Adding equi-molar concentration of competing ions to single Pb(II) and Zn(II) ion systems changed the partitioning behavior of both metal ions relative to single-metal-ion systems, especially at low concentrations. The extent of competitive sorption effects was found to depend on the concentration of metal ions and the type of reactive surfaces. At high metal-ion concentrations, there were no apparent competitive effects observed for Pb(II) or Zn(II) in both the α -Al₂O₃ (1-102) and α -Fe₂O₃ (0001)/biofilm systems. Competitive effects were found to be more pronounced at lower metal concentrations. Adding an equi-molar concentration of Zn(II) to the Pb(II)/*S. oneidensis*/ α -Al₂O₃(1-102)/water system caused Pb(II) to partition more into the biofilms and less onto the α -Al₂O₃ (1-102) surfaces at low metal-ion concentrations. In contrast, adding equi-molar concentration of Pb(II) to the Zn(II)-containing systems caused Zn(II) to partition more into biofilms and significantly less onto α -Fe₂O₃ (0001) surfaces. Therefore, Pb(II) outcompeted Zn(II) on α -Fe₂O₃ (0001) surfaces, whereas Zn(II) outcompeted Pb(II) on α -Al₂O₃ (1-102) surfaces. For both the Pb(II)- and Zn(II)-containing systems, single-metal-ion partitioning data did not predict the observed

partitioning behavior in mixed-metal-ion systems at low metal concentrations. This study strongly suggests that competitive sorption effects in multi-metal-ion systems must be carefully studied to predict accurately the mobility of metal ions in complex natural environments.

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Tables and Figures

Effect of biofilm coatings at metal-oxide/water interfaces II: Competitive sorption between Pb(II) and Zn(II) at *Shewanella oneidensis*/metal-oxide/water interfaces

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Table 1

Results of LP-XSW-FY data analysis for Pb(II) and Zn(II) partitioning at *S. oneidensis* MR-1 biofilm-coated α -Al₂O₃ (1-102) and α -Fe₂O₃ (0001) surfaces as a function of equi-molar Pb and Zn initial concentrations ranging from 10⁻⁴ to 10⁻⁷ M, pH = 6.0 ± 0.05, reaction time = 3 hr.

Substrate	Initial [Pb(II)]	Initial [Zn(II)]	Pb(II) partitioning			Zn(II) partitioning		
			% at surface ^a	% in biofilm ^b	STDEV	% at surface ^a	% in biofilm ^b	STDEV
α -Al ₂ O ₃ (1-102)	10 ⁻⁴ M	10 ⁻⁴ M	<1	>99	<1	<1	>99	<1
α -Al ₂ O ₃ (1-102)	10 ⁻⁵ M	10 ⁻⁵ M	<1	>99	<1	<1	>99	<1
α -Al ₂ O ₃ (1-102)	10 ⁻⁶ M	10 ⁻⁶ M	5	95	1.5	7	93	3.8
α -Al ₂ O ₃ (1-102)	10 ⁻⁷ M	10 ⁻⁷ M	13	87	2.5	28	72	4.5
α -Fe ₂ O ₃ (0001)	10 ⁻⁵ M	10 ⁻⁵ M	<1	>99	<1	<1	>99	<1
α -Fe ₂ O ₃ (0001)	10 ⁻⁶ M	10 ⁻⁶ M	23	77	3.5	3	97	0.5
α -Fe ₂ O ₃ (0001)	10 ⁻⁷ M	10 ⁻⁷ M	47	53	5.0	<1	>99	<1

^a Average percentage distribution at the metal-oxide surface from replicates.

^b Average percentage distribution in the biofilms from replicates.

Table 2

Site densities of various types of surfaces and their binding affinities to Pb(II) and Zn(II).

Type of surface	Site type	Site density	Pb(II) binding affinity (log <i>K</i>)	Zn(II) binding affinity (log <i>K</i>)
<i>S. oneidensis</i> MR-1 (Ha et al., 2010)	Carboxyl	1.4×10^{-4} mol/g _{wet}	4.6 ^a	3.6 ^a
	Phosphoryl	4.1×10^{-5} mol/g _{wet}	4.1 ^a	3.7 ^a
<i>Enterobacteriaceae</i> (Ngwenya et al., 2003)	Carboxyl	6.25×10^{-5} mol/g _{wet} ^b	3.9 (±0.8)	3.3 (±0.1)
	Phosphoryl	2.75×10^{-5} mol/g _{wet} ^b	5.0 (±0.9)	5.1 (±0.1)
<i>A. macleodii</i> subsp <i>fijiensis</i> (Loaec, 1997)	EPS	-	5.2	4.4
α -Al ₂ O ₃ (1-102) (Templeton et al., 2001)	Strong	2.5×10^{-6} mol/m ^{2c}	6.0	-
	Weak	5.1×10^{-6} mol/m ^{2c}	3.55	-
γ -Al ₂ O ₃ (Davis and Leckie, 1978)	Monodentate ^d	$\text{AlO}^- + \text{Me}^{2+} = \text{AlO}^- \text{Me}^{2+}$	6.4 ^e	-
	Monohydroxyl complex ^d	$\text{AlO}^- + \text{MeOH}^+ = \text{AlO}^- \text{MeOH}^+$	8.7-10.2 ^f	10.1 ^e
α -Fe ₂ O ₃ (0001) (Templeton et al., 2001)	Strong	3.1×10^{-6} mol/m ^{2c}	6.65	-
	Weak	6.3×10^{-6} mol/m ^{2c}	3.5	-
$\text{Fe}_2\text{O}_3 \cdot \text{H}_2\text{O}(\text{am})$ (Davis and Leckie, 1978)	Monodentate ^d	$\text{FeO}^- + \text{Me}^{2+} = \text{FeO}^- \text{Me}^{2+}$	6.9 ^e	-
	Monohydroxyl complex ^d	$\text{FeO}^- + \text{MeOH}^+ = \text{FeO}^- \text{MeOH}^+$	11.1 ^e	9.4 ^e

^a Averaged log *K* from Ha et al. (2010).

^b Site concentrations are converted to g_{wet} using a conversion factor of 8:1 for wet to dry mass ratio.

^c Modified site densities from Wang et al., submitted.

^d Me represents as divalent metal ions such as Pb(II) or Zn(II).

^e At metal-ion concentration of 5×10^{-7} M.

^f The values vary as a function of Pb(II) concentration ranging from 2.9×10^{-4} to 5×10^{-7} M.

Table 3

Cation radius, hydrated cation radius, water exchange rate, and diffusion coefficients for Zn(II), Pb(II), and Cu(II) in a simple electrolyte solution and in Na-PAA solution.

Metal ions	Ionic radius octahedral coordination (Å) ^a	Hydrated cation radius (Å) ^b	Water exchange rate (s) ^c	Diffusion coefficient in 0.02M KCl (cm ² /s) ^d	Diffusion coefficient in 0.036 M Na-PAA (cm ² /s) ^e
Zn	0.74	4.30	7x10 ⁷	7.1x10 ⁻⁶	2.4 (±0.3) x10 ⁻⁹
Pb	1.19	4.05	7x10 ⁹	9.4 x10 ⁻⁶	-
Cu	0.73	2.0 (2.3) ^f	1x10 ⁹	12.4x10 ^{-6g}	6 (±3) x 10 ⁻¹¹

^a From Shannon (1976).

^b From Nightingale (1959).

^c From Stumm and Morgan (1996).

^d From Wang (1954), T = 25°C.

^e From Kojima and Fujiwara (1970), T = 22.5°C.

^f From Akesson et al. (1992), the value in parenthesis is the Jahn-Teller distortion radius.

^g From Ribeiro et al. (2005).

Fig. 1. X-ray reflectivity and LP-XSW-FY profiles for Pb(II) and Zn(II) partitioning at *S. oneidensis* MR-1 biofilm-coated α - Al_2O_3 (1-102) surfaces as a function of equi-molar Pb and Zn initial concentrations ranging from 10^{-4} to 10^{-7} M, pH = 6.0 ± 0.05 , reaction time = 3 hr: (a) Pb(II) FY spectra; (b) Zn(II) FY spectra. Black filled circles represent normalized x-ray reflectivity data, grey solid lines represent normalized x-ray reflectivity fits. Red open circles represent FY data for Pb, red solid lines represent FY fit for Pb, blue open circles represent FY data for Zn, blue solid lines represent FY fit for Zn.

Fig. 2. The effect of competing ions on metal-ion partitioning at *S. oneidensis* MR-1 biofilm-coated α -Al₂O₃ (1-102) surfaces as a function of equi-molar Pb(II) and Zn(II) initial concentrations ranging from 10⁻⁴ to 10⁻⁷ M, pH = 6.0 $\pm$ 0.05, reaction time = 3 hr: (a) the effect of Zn(II) on Pb(II); (b) the effect of Pb(II) on Zn(II).

Fig. 3. X-ray reflectivity and LP-XSW-FY profiles for Pb(II) and Zn(II) partitioning at *S. oneidensis* MR-1 biofilm-coated α -Fe₂O₃ (0001) surfaces as a function of equi-molar Pb(II) and Zn(II) initial concentrations ranging from 10^{-5} to 10^{-7} M, pH = 6.0 ± 0.05 , reaction time = 3 hr: (a) Pb(II) spectra; (b) Zn(II) spectra. Black filled circles represent normalized reflectivity data, grey solid lines represent normalized reflectivity fits. Red open circles represent FY data for Pb, red solid lines represent FY fit for Pb, blue open circles represent FY data for Zn, blue solid lines represent FY fit for Zn.

Fig. 4. The effect of competing ions on metal-ion partitioning at *S. oneidensis* MR-1 biofilm-coated α -Fe₂O₃ (0001) surfaces as a function of equi-molar initial Pb and Zn concentrations ranging from 10^{-5} to 10^{-7} M, pH = 6.0 ± 0.05 , reaction time = 3 hr: (a) the effect of Zn(II) on Pb(II); (b) the effect of Pb(II) on Zn(II).