**Effect of biofilm coatings at metal-oxide/water interfaces I:
Pb(II) and Zn(II) partitioning and speciation at *Shewanella
oneidensis*/metal-oxide/water interfaces**

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Abstract

Microbial biofilms are often present as coatings on metal-oxide surfaces in natural and industrial environments and may induce significant changes in the partitioning behavior and speciation of aqueous metal ions, which in turn can impact their transport and fate. In this study, long-period x-ray standing wave-fluorescence yield (LP-XSW-FY) spectroscopy was used to measure under *in situ* conditions the partitioning of aqueous Pb(II) and Zn(II) between multilayer *Shewanella oneidensis* MR-1 biofilms and highly polished, oriented single-crystal surfaces of α -Al₂O₃ and α -Fe₂O₃ as a function of metal-ion concentration and time at pH 6.0. We show that after 3-hrs exposure time, Pb(II) binds preferentially to the α -Al₂O₃ (1-102) and α -Fe₂O₃ (0001) surfaces at low Pb concentration ([Pb] = 10⁻⁷ M) and then increasingly partitions into the biofilm coatings at higher concentrations (10⁻⁶ to 10⁻⁴ M). In contrast, Zn(II) partitions preferentially into the biofilm coating for both surfaces at all Zn concentrations studied (10⁻⁷ to 10⁻⁴ M). In comparison, the α -Al₂O₃(0001) surface has a low affinity for both Pb(II) and Zn(II), and the biofilm coatings are the dominant sink for both ions. These findings suggest that in the presence of *S. oneidensis* biofilm coatings, α -Al₂O₃(0001) is the least reactive surface for Pb(II) and Zn(II) compared to α -Al₂O₃(1-102) and α -Fe₂O₃(0001). They also show that Zn(II) has a lower affinity than Pb(II) for reactive sites on α -Al₂O₃(1-102) and α -Fe₂O₃(0001) at [Me(II)] of 10⁻⁷ M; at 10⁻⁵ M, the bulk of the metal ions partition into the biofilm coatings. At longer exposure times (20-24 hr), both Pb(II) and Zn(II) increasingly partition to the metal-oxide surfaces at [Me(II)] = 10⁻⁵ M and pH 6.0, indicating possible reaction/diffusion-controlled sorption processes. Pb L_{III}-edge and Zn K-edge grazing-incidence extended x-ray absorption fine structure (GI-EXAFS) measurements suggest that both Pb(II) and Zn(II) ions may be complexed by carboxyl groups in *S. oneidensis* biofilms after 3-hr exposure at pH 6.0 and [Me(II)] = 10⁻⁵ M. In contrast with *Burkholderia cepacia*, which was used in our previous studies of monolayer biofilm-coated metal-oxide surfaces (Templeton et al., 2001), *S. oneidensis* MR-1 forms relatively thick biofilm coatings (6-20 μ m) that are rich in reactive functional groups and are expected to dominate metal-ion adsorption. Our results show that even thick and highly reactive biofilms like *S. oneidensis* do not cause much change in the intrinsic

64 chemical reactivities of the underlying metal-oxide surfaces with respect to aqueous
65 Pb(II) and Zn(II) and don't block reactive sites on the metal-oxide surfaces; instead they
66 reduce the rate of Pb(II) and Zn(II) sorption onto these surfaces.

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1. INTRODUCTION

The mobility and geochemical cycling of metal ions in natural and contaminated aquatic systems are impacted by a number of surface chemical processes such as complexation, precipitation, and redox transformations at mineral/water interfaces. These processes are often complex and strongly dependent on pH, pE, metal-ion concentration, and the type and concentration of background electrolytes (Boily and Fein, 1996; Brown et al., 1999). This scenario is further complicated by the presence of microbes, which are found in almost every environment on Earth (e.g., Ghiorse and Wilson, 1988; Reysenbach and Shock, 2002). The majority of microbes in nature exist in the form of biofilms (Donlan and Costerton, 2002), which are bacterial communities living in hydrated films of extracellular polymeric substances (EPS) that often attach to mineral surfaces (e.g., Costerton et al., 1995; Beveridge et al., 1997; Barker et al., 1998; Davey and O'Toole, 2000; Templeton et al., 2001). In addition to hosting microbial colonies, mineral surfaces can also be coated extensively by various forms of natural organic matter (NOM) especially under acidic and neutral pH conditions where many mineral surfaces are positively charged due to their high pH_{PZC} values (e.g., Mayer, 1994; Ransom et al., 1997; Barker et al., 1998; Kaiser and Guggenberger, 2000; Ganor et al., 2009). These coatings exhibit large surface areas and contain abundant reactive anionic functional groups, which are expected to induce significant changes in the properties of mineral surfaces and influence metal-ion cycling by blocking the reactive sites on the mineral surface, modifying the surface charge properties of mineral/water interfaces, or providing new sorption sites in bacteria or NOM (e.g., Fein et al., 1997, Templeton et al., 2001, Yoon et al., 2005).

Experimental studies of mineral surface interactions of aqueous metal ions are typically carried out in systems with clean mineral surfaces (e.g., Bargar et al., 2004) or on bacterial/biofilm surfaces (e.g., Ferris et al., 1989; Fein et al., 1997). Relatively few attempts have been made to evaluate the effects of biofilm or fungal coatings on mineral reactivity and metal-ion cycling at the molecular level under *in situ* conditions (Templeton et al., 2001; Templeton et al., 2003b; Bonneville et al., 2009). This is in part due to the complex nature of these systems, and the fact that only a few techniques can

determine the positions and speciation of ions at biofilm/mineral interfaces at ambient temperature and pressure in the presence of a fluid phase. As a result, there is little direct information on the molecular-level speciation and distribution of metal ions at mineral/microbe/water interfaces or on the structure and composition of adsorption complexes and/or precipitates in these systems. Therefore, the effects of biofilm coatings on chemical reactions occurring at mineral/water interfaces and on trace metal cycling are poorly understood. Here we assess these effects using synchrotron-based long period x-ray standing wave fluorescence yield (LP-XSW-FY) spectroscopy, which is very sensitive to the distribution of ions between a solid surface and an overlying microbial biofilm.

The reactivity of a surface, whether biological or inorganic, is dependent on a number of fundamental surface properties including composition, metal(loid) affinity, abundance of reactive binding sites, and electrostatic properties of the electrical double layer (Brown et al., 1999; Brown et al., 2007). In the case of metal oxides, advances in synchrotron-based x-ray absorption spectroscopy and surface x-ray scattering methods have provided a new understanding of the surface compositions and structures of orientated, hydrated single-crystal α -Al₂O₃ and α -Fe₂O₃, including the different types of reactive surface sites (e.g., Bargar et al., 1997a; Trainor et al., 2004). Single crystal α -Al₂O₃ and α -Fe₂O₃ surfaces are reported to have site densities of functional groups ranging from 1.1 to 9.4 $\mu\text{mol}/\text{m}^2$, and Pb(II) binding affinities ranging from 2.7 to 6.65 (Templeton et al., 2001). For bacteria, there is even more complexity in the types of outer membrane and EPS functional groups including carboxyl, phosphoryl, hydroxyl, amine, and sulfhydryl groups for different types of microbes (e.g., Ferris et al., 1989; Fein et al., 1997; Wei et al., 2004). Ginn and Fein (2008) reported the average site densities of functional groups ranging from $1.0 (\pm 0.17) \times 10^{-4}$ to $4.6 (\pm 1.8) \times 10^{-5}$ moles/g_{wet}, and average Pb(II) binding affinities ranging from 3.3 to 7.9 for five different species of bacteria representing a wide genetic and habitat diversity. Site densities and metal-ion binding affinities of various functional groups present on bacterial or mineral surfaces are important factors in determining how strongly the metal ions interact with these surfaces.

Surface charge also has an impact on the electrostatic interactions of metal ions with microbial and mineral surfaces. The surface charge of bacteria is negative in most cases

under neutral pH conditions (Yee et al., 2004). For example, the surfaces of planktonic cells of *Shewanella oneidensis* MR-1 have an isoelectric point (IEP) of approximately 4.0 (Ha et al., 2010). In contrast, the surface charges of Fe- and Al-(oxyhydr)oxides are typically positive at neutral pH, with IEP values in the range of 8-9 for powders and nanoparticles and in the range of 4-6 for single-crystal surfaces (Parks, 1965; Sverjensky, 1994; Franks and Meagher, 2003, Wang et al., in press). Such differences in surface charge can impact the electrostatic interactions of metal ions with microbial and mineral surfaces. Therefore, bacteria and biofilms can potentially compete with mineral surfaces for metal-ion sorption through reactions with the abundant functional groups present in bacterial cell walls and associated EPS, and/or result in more negatively charged mineral surfaces. An understanding of these properties is essential for determining or predicting relative differences in overall adsorption behavior of metal ions in microbe/metal-oxide/water systems, particularly the competition between microbe and mineral surfaces for aqueous metal ions.

In addition to the intrinsic abiotic properties of microbes and mineral surfaces, the complex metabolic activities of microbial colonies such as biofilms can create microenvironments in which pH, Eh, and ion concentrations may differ substantially from bulk solution values (Barker et al., 1998; Warren and Haack, 2001). Such differences can enhance (or inhibit) metal-ion adsorption on mineral surfaces and result in the formation of biominerals (Konhauser, 1998; Morin et al., 2003; Templeton et al., 2003c). For example, *Burkholderia cepacia* causes biomineralization of Pb(II) in the form of highly insoluble pyromorphite ($(\text{Pb}_5(\text{PO}_4)_3(\text{OH}, \text{Cl}, \text{F}))$) for metabolically active cells, even at Pb concentrations in the bulk solution well below saturation with respect to pyromorphite (Templeton et al., 2003c). Templeton et al. (2003c) suggested that cell-associated phosphates form highly insoluble metal phosphates under toxic metal stresses, thus sequestering Pb and reducing its toxicity. Such processes may dramatically alter the solubility, mobility, and toxicity of Pb and other potential environmental contaminants.

Synchrotron-based LP-XSW-FY spectroscopy is a unique *in situ* probe for examining the distribution of trace elements between a thin-film coating and an underlying substrate (Templeton et al., 2001; Bedzyk and Cheng, 2002; Trainor et al., 2006). Templeton et al. (2001) first used the *in situ* LP-XSW-FY technique to probe Pb(II) partitioning and

speciation on *Burkholderia cepacia* biofilm-coated single-crystal surfaces of α - Al_2O_3 (0001) and (1-102), and α - Fe_2O_3 (0001). They found that at $[\text{Pb}_{(\text{aq})}] = 1 \text{ } \mu\text{M}$, pH 6, and an ionic strength of 0.005 M, Pb(II) adsorption at *B. cepacia*/ α - Al_2O_3 (1-102) and *B. cepacia*/ α - Fe_2O_3 (0001) interfaces is dominated by complexation to high affinity metal-oxide surface sites despite an excess of potentially available binding sites in the overlying biofilms. The biofilm became a sink for Pb(II) only when the reactive metal-oxide surface sites were effectively populated by Pb(II) with increasing Pb(II) solution concentration ($> 10^{-5} \text{ M}$). In contrast, Pb(II) partitioned mostly into the biofilm for the *B. cepacia* biofilm/ α - Al_2O_3 (0001) system at $[\text{Pb}_{(\text{aq})}] \geq 10^{-6} \text{ M}$. This study was the first to demonstrate that *monolayer* biofilm coatings do not block the reactive sites of metal-oxide surfaces under the conditions specific to the experiments. However, as discussed in more detail in Section 4.1, the site densities and metal-ion binding affinities of different bacterial species exhibit significant variations, although a universal proton and metal-ion adsorption model for bacteria has been proposed (e.g., Yee and Fein, 2001; Johnson et al., 2007; Ginn and Fein, 2008). Therefore, the conclusion reached by Templeton et al. (2001) cannot be generalized to other mineral/microbe/water interfaces without further testing of various types of biofilm-coated metal-oxide systems.

In addition, biofilms are well known to have functional, structural, and chemical heterogeneities. One major difference between planktonic cells and biofilm cells is the extensive polymer network (i.e. EPS) associated with the biofilms. For example, biofilms are much more resistant to metal toxicity or antibiotics compared to planktonic cells (Donlan and Costerton, 2002). A kinetic adsorption experiment on free cells and well-mixed systems showed that metal-ion adsorption can reach equilibrium in less than 15 minutes to an hour (Fein et al., 1997). However, this time frame could be significantly different for multilayer biofilm structures. For example, antibiotic agents can kill free planktonic cells in 15 seconds compared to 20 minutes for biofilms (Donlan and Costerton, 2002). It has been reported that Ni(II) and Zn(II) diffuse relatively slowly into biofilms of *Escherichia coli* and *Pseudomonas aeruginosa*, thus increasing their resistance to toxic metal species (Teitzel and Parsek, 2003; Hu et al., 2005). These findings suggest that there is a need to study the kinetics of metal diffusion in biofilm/metal-oxide systems over longer reaction times.

In the present study we used synchrotron-based LP-XSW-FY spectroscopy to determine quantitatively under *in situ* conditions the partitioning of aqueous Pb(II) and Zn(II) between *Shewanella oneidensis* MR-1 biofilm coatings and underlying, oriented single-crystal α -Al₂O₃ and α -Fe₂O₃ substrates as a function of metal ion concentration, reaction time, and pH. When grown on highly polished and oriented single-crystal surfaces of α -Al₂O₃ and α -Fe₂O₃, as done here, *S. oneidensis* forms multi-layer biofilms several microns thick, in contrast with the earlier study where *B. cepacia* formed monolayer biofilms with an average thickness of $\sim 1\mu\text{m}$ (Templeton et al., 2001). We also carried out grazing-incidence EXAFS spectroscopy on Pb(II) and Zn(II) sorbed on biofilm-coated α -Al₂O₃ (1-102) surfaces to determine their local coordination environments and mode of binding at these complex interfaces.

2. MATERIALS AND METHODS

2.1. Choice of systems

S. oneidensis MR-1, a facultative Gram-negative metal-reducing bacterium, is the most diverse respiratory organism found to date (Arnold et al., 1990; Heidelberg et al., 2002; Hau and Gralnick, 2007). It can interact with Fe- and Mn-containing minerals and various redox-sensitive elements such as As and Se, and thus plays a critical role in mineral dissolution and heavy-metal(loid)/pollutant sequestration and (im)mobilization. This bacterium is widely used in biotechnology and microbial fuel cells, and was chosen as a potential microorganism by the Department of Energy for bioremediation (Heidelberg et al., 2002). In addition, a complete genome sequence is known for this microorganism, and genetic and biochemical manipulations are being conducted routinely (Heidelberg et al., 2002). In particular, *S. oneidensis* MR-1 is known to form biofilms, and their properties have been well studied (Thormann et al., 2004; Thormann et al., 2005). Information on site densities and metal-ion binding constants of various functional groups on *S. oneidensis* MR-1 cell surfaces is available for planktonic *S. oneidensis* MR-1 cells (Ha et al., 2010). These unique properties coupled with an extensive knowledge of this species make *S. oneidensis* a good model microorganism for the present study.

The metal oxides used in this study consist of highly polished and oriented single-crystal surfaces of α -Al₂O₃ and α -Fe₂O₃. Fe- and Al-(oxyhydr)oxides are common in nature, often exhibit small particle size and high surface areas, and are among the most reactive mineral surfaces in contact with surface and ground waters and soils (Hsu, 1989; Cornell and Schwertmann, 2003). Thus, they are potentially important sorbents for metal ions in the environment. Because their surface structures and types of functional groups are relatively well-known (Eng et al., 2000; Trainor et al., 2002; Trainor et al., 2004), orientated single-crystal α -Al₂O₃ and α -Fe₂O₃ surfaces serve as good model mineral substrates.

Pb(II) and Zn(II) were chosen as the aqueous cations in this study. Pb is a toxic heavy metal found at trace levels in natural environments (averaging 14 ppm (by weight) in crustal rocks, and 35 ppm in soils) and as a major pollutant in some anthropogenically perturbed environments (Nriagu, 1978). Zn(II) is also present at trace levels in Earth materials (averaging 60 ppm by weight in soils) (Shacklette and Boerngen, 1984) and is generally considered to be a nutrient because it is an essential component of biological systems; however, it can be toxic when present at high concentration (>100-300mg Zn/day vs. Recommended Dietary Allowance of 15mg Zn/day, Fosmire, 1990). There have been extensive studies of Pb(II) and Zn(II) adsorption on metal-oxide surfaces (e.g., Bargar et al., 1997b; Trainor et al., 2000; Bargar et al., 2004) as well as on bacteria and fungal cells (e.g., Sarret et al., 1998; Templeton et al., 2001; Templeton et al., 2003a; Templeton et al., 2003c; Guine et al., 2006; Toner et al., 2005; Claessens and Van Cappellen, 2007; Boonfueng et al., 2009; Tournay et al., 2009; Ha et al., 2010). The findings from these studies are particularly useful in developing an understanding of metal-ion partitioning behavior and speciation at complex microbe/metal-oxide/water interfaces.

2.2. Crystal preparation

The metal-oxide substrates used in these experiments were commercially available, highly polished α -Al₂O₃ (0001) and (1-102) single crystals (Saint-Gobain Crystals & Detectors Co.) and natural hematite α -Fe₂O₃ (0001) (variety specularite) single crystals from Bahia, Brazil. The α -Fe₂O₃ crystals were prepared using a chemical-mechanical

cleaning and polishing procedure that results in surfaces of sufficient quality for x-ray reflectivity and LP-XSW-FY studies (Trainor et al., 2004; Tanwar et al., 2007). Prior to the growth experiments, all substrates were carefully cleaned with acetone, then washed in $10^{-3.5}$ M sodium hydroxide for 20 minutes, and subsequently washed in 10^{-2} M nitric acid for an hour. Each chemical washing step was followed by multiple rinses with MilliQ water. The commercial α -Al₂O₃ crystals were also washed as described above and then baked at 350°C for 4 hr to minimize excess carbon on the surfaces. The cleaning procedure was repeated as necessary until the organic (adventitious) carbon content at the crystal surfaces was less than ~10% and other metal contents were undetectable as determined by x-ray photoelectron spectroscopy (XPS) (Surface Science S-Probe, monochromatic Al K α radiation). Atomic force microscopy (AFM) (Veeco Multimode Scanning probe microscope) conducted on representative α -Al₂O₃ (0001) and (1-102), and α -Fe₂O₃ (0001) surfaces showed that the surface roughness of these crystals is generally less than 5 Å, which makes them suitable for LP-XSW-FY and GI-XAFS spectroscopy studies. The cleaned metal-oxide substrates were then glued using epoxy onto cylindrical flow reactors, and the entire assembly (flow reactors with all the metal-oxide substrates and associated Tygon tubing) was autoclaved for 20 minutes before inoculation with *S. oneidensis* MR-1 bulk cells. A schematic of the experimental set up for biofilm growth is shown in Fig. 1.

2.3. Biofilm growth

Shewanella oneidensis MR-1 biofilms were grown aerobically on the single-crystal substrates in a flow-through reactor equipped with inlet and outlet for the introduction and removal of a nutrient medium composed of 1.27 mM K₂HPO₄, 0.73 mM KH₂PO₄, 5 mM NaHEPES, 2 mM NaHCO₃, 9 mM (NH₄)₂SO₄, 150 mM NaCl, and 0.64 mM CaCl₂. The pH of the medium was adjusted to 7.0 for optimum growth condition, and 0.5 mM sodium pyruvate was used as a carbon and electron source. All media were freshly prepared and autoclaved. Pyruvate and CaCl₂ were added under sterile conditions after the autoclaved medium solution cooled down to avoid pyruvate degradation and calcium phosphate precipitation, respectively.

S. oneidensis MR-1 cultures were grown on plates in contact with Luria-Bertani broth (LB) gel at 30°C for 24 hrs, and then at room temperature for 24 hrs. Subsequently, 2 L flasks, each containing 200 ml of the LB medium, were inoculated and incubated at 30°C under aeration, while constantly being shaken at 210 rpm. After the culture reached the late exponential growth phase (16 hr), the culture was transferred into a sterilized cylindrical flow reactor. The cells were allowed to attach to the metal-oxide surfaces for 1 hr, and then fresh sterile medium was continuously pumped across the surface and through the reactor. Extreme care was taken to avoid air bubbles and possible shear of the biofilm from the crystal surfaces due to movement of the assembly during biofilm growth. A constant, low-pulse flow was maintained using a peristaltic WATSON/MARLOW pump (model 205S/CA, Wilmington, MA), with an applied flow rate of 0.4 ml min⁻¹. The entire assembly was placed in a 30°C constant-temperature room for optimum biofilm growth under aerobic conditions. After a specific time period (typically 10 days for samples used in LP-XSW-FY/GI-XAFS measurements), the flow-through reactor was flushed overnight under sterile conditions with fresh autoclaved minimum medium (200 µM CaCl₂, 150 µM MgSO₄, 90 µM (NH₄)₂SO₄, 150 µM KNO₃, 10 µM NaHCO₃, 5 µM KH₂PO₄) in order to remove the relatively rich initial nutrient medium. The substrates with biofilms were carefully removed from each reactor under sterile conditions and rinsed three consecutive times in fresh sterile 0.01 M NaNO₃ solution in order to remove excess ions from the medium adsorbed on the cells and substrate surface.

2.4. Confocal laser scanning microscopy analysis of biofilm growth on metal-oxide substrates

Biofilms grown on the metal-oxide substrates using *gfp*-labeled *S. oneidensis* MR-1 cells were visualized *in situ* using confocal laser scanning microscopy (CLSM), and the three dimensional structures of the biofilms and other important parameters such as surface coverage, biomass, and biofilm thickness were measured. The biofilms grown on the metal-oxide substrates after 5, 10, and 20 days were observed using an upright Zeiss LSM510 CLSM confocal microscope (Carl Zeiss, Jena, Germany) equipped with 20x/0.5 W Achroplan, and 60x/1.2 W C-Apochromat objectives. Each sample was observed first

under the microscope with bright field or fluorescence to check the biofilm coverage on the metal-oxide substrates. Biofilms were scanned in 0.5 μm z-intervals, and fluorescence images were used to reconstruct a 3D architecture of the biofilms on the substrates. The average biomass, biofilm thickness, and roughness parameters were obtained from at least 10 different positions of multiple independent crystal samples with the same growth conditions. To display the 3D structure of the biofilm, CLSM images were processed using the IMARIS software package (Bitplane AG, Zürich, Switzerland) and Adobe Photoshop. Biofilm parameters such as biomass, average biofilm thickness, and roughness were quantified with the program COMSTAT (Heydorn et al., 2000). Based on a study of biofilm growth at three metal-oxide surfaces as a function of time, we found that 10-day growth produced relatively continuous and smooth surface coverage for all three metal-oxide surfaces as shown in Fig. 2 and this growth time was chosen for the samples used in subsequent LP-XSW-FY and GI-XAFS measurements (see Appendix A1 and Figs. A1-A2 for 5-day and 20-day biofilm growth periods).

2.5. Preparation of Pb(II)- and Zn(II)-exposed biofilm/metal-oxide samples

Reagent grade $\text{Pb}(\text{NO}_3)_2$ and $\text{Zn}(\text{NO}_3)_2$ (J.T. Baker) were used to prepare metal stock solutions. Rinsed biofilm-coated single-crystal samples were gently immersed in 35 ml of Pb(II)- or Zn(II)-containing aqueous solution of desired concentration at $\text{pH } 6.00 \pm 0.05$. A N_2 -degassed 0.01 M NaNO_3 aqueous solution was used as the background electrolyte for all the metal-ion-containing aqueous solutions. Extreme care was taken to keep the biofilm structure intact during the experiments. All experiments were performed in aqueous solutions undersaturated with respect to any solid phase, as checked by calculations using the visual MINTEQ computer code and corresponding database (Gustafsson, 2007). A metal exposure time of 3 hr was initially chosen for the experiments that measured Pb(II) and Zn(II) partitioning as a function of metal-ion concentrations to compare the results with previous work (Templeton et al., 2001). Shorter (20-30 min) and longer time (20-24 hr) exposures were tested for selected metal-oxide surfaces and metal concentrations to study possible kinetic effects. No solid precipitation was observed in the solution phase during these exposures. The solution pH was checked frequently during metal exposure experiments and any deviation from pH

6.0 was adjusted back to this value by adding small volumes of NaOH or HNO₃ solution (0.001 to 0.01 M). To probe the effect of metal-ion concentration on metal-ion partitioning/speciation between the biofilm and the metal-oxide substrate, new batch solutions were prepared with initial Pb(II) or Zn(II) concentrations varying from 10⁻⁴ M to 10⁻⁷ M. For each experimental condition, a fresh biofilm-coated metal-oxide sample was used in a fresh 35 ml metal-ion-containing aqueous solution. After exposure to metal ions for the selected reaction time, the biofilm-coated metal-oxide samples were carefully withdrawn from the solution, and then mounted in an enclosed sample holder for LP-XSW-FY and GI-XAFS measurements. Humidified He gas was pumped through the sample holder to keep the sample under hydrous conditions during the course of LP-XSW-FY and GI-XAFS measurements.

2.6. LP-XSW-FY and grazing-incidence EXAFS measurements

Synchrotron-based LP-XSW-FY provides a sensitive, element-specific and spatially resolved probe of element partitioning between biofilm or organic coatings and underlying solid surfaces and is suitable for the length scales typical of such coatings (Templeton et al., 2001; Bedzyk and Cheng, 2002; Trainor et al., 2006). In brief, x-ray standing waves are generated by the superposition of coherently coupled incident and reflected beams. The constructive interferences of these two beams create the antinodes of the XSW, and the positions of the antinodes relative to the reflecting substrate can be varied by changing the angle of incidence or energy of the x-ray beam (Bedzyk and Cheng, 2002). X-ray fluorescence of a specific element is generated as the antinodes of the x-ray standing waves interact with the element at different heights above the biofilm-mineral interface. The fluorescence yield of each element present in the system at concentrations > 10⁻⁸ M, and sometimes lower, can be monitored during this process as a function of incident x-ray angle using an energy dispersive fluorescence detector. The LP-XSW-FY data can be fit using a general scattering model, which allows quantification of the vertical distribution of specific elements.

LP-XSW-FY measurements were performed on insertion device beamline 13-ID-C at GeoSoilEnviroCARS (GSECARS) at the Advanced Photon Source (APS) using a general-purpose diffractometer in horizontal scattering geometry. Fig. 3 shows a

schematic of the LP-XSW-FY experimental configuration (see the experimental set-up in Appendix Fig. A3). Monochromatic x-rays were produced with a liquid N₂-cooled Si(111) monochromator illuminated with x-rays produced by the APS undulator "A" set at its 3rd harmonic. The monochromatic x-rays were then focused using a pair of 1 m long dynamically figured Si mirrors arranged as a Kirkpatrick-Baez (KB) pair, allowing the size and divergence of the horizontal and vertical beam to be adjusted independently. Using the KB mirror pair, the beam was collimated in both the vertical and horizontal directions. In the vertical the full 1 mm tall, collimated beam was used, and in the horizontal the 1.5 mm wide collimated beam was reduced in size to 15 to 20 μm by a tantalum slit 1 m upstream from the sample. This x-ray optics configuration provided optimal coupling of the APS undulator source to the small grazing-incidence acceptance aperture of the sample with its surface normal in the horizontal plane. Samples were mounted in a Teflon sample holder covered with a thin Mylar film purged by humidified He gas during our experiments. The incidence angle of the highly collimated (divergence less than 0.001°) x-ray beam was scanned between 0.0 and 0.5° for x-ray reflectivity measurements, while the incident (I_0) and reflected (I_1) x-rays were monitored through N₂-filled gas ionization chambers. Fluorescence yield data were collected at 14 keV incident x-ray energy. Fluorescence spectra were acquired using a 4-element silicon drift detector (SII NanoTechnology, Vortex-ME4) coupled to digital x-ray processor electronics (X-ray Instrumentation Associates, xMAP). The detector array was located in the horizontal plane and 90° to the incident beam in order to suppress the charge scattering background from the sample. Each of the four detector elements was fit with a collimator so that it could only "see" the x-ray foot print at the center of the sample and thus block scatter and fluorescence x-rays from other parts of the sample and environment from being collected by the detector.

The fluorescence yield spectra from each of the four detector elements were averaged and corrected for detector dead time. In the current study, we used weight-averaged values of Pb $L\alpha_{1,2}$ and Zn $K\alpha_{1,2}$ as Pb $L\alpha$ and Zn $K\alpha$, respectively. The total areas of Pb $L\alpha$ and Zn $K\alpha$ peaks were fit using a Gaussian line shape on a linear background. Repetitive scans, each taking 30 minutes, of LP-XSW-FY spectra with the x-ray beam centered at the same positions on selected samples showed similar profiles. These

observations indicate that x-ray beam exposure of the biofilm-coated metal-oxide samples did not cause detectable changes in the distribution of Pb(II) or Zn(II) during the LP-XSW-FY measurement or other detectable sample damage. Nonetheless, in order to avoid undetectable changes that might be caused by the x-ray beam, the initial alignment was performed at one position for a sample, and each subsequent LP-XSW-FY profile was then collected at a fresh spot after quick realignment to reduce beam exposure. The sample was moved up or down in position at least 1 mm away from the initial alignment position for previous scans, and at least three replicates were collected for each sample. The typical sample size was 1 cm × 1.2 cm, and the biofilms covered the crystal entirely.

LP-XSW-FY and GI-XAFS measurements were also conducted at the Stanford Synchrotron Radiation Lightsource (SSRL) on beamline 11-2 using a grazing-incidence spectrometer in vertical scattering geometry (see Appendix Fig. A4 for the experimental set up). X-rays were focused using a Pt-coated mirror, and an incident beam energy of 14 keV was selected using a liquid nitrogen-cooled Si(220) double-crystal monochromator ($\phi = 90^\circ$) and collimated to 1 mm horizontal by 20 μm vertical. The incidence angle was scanned from 0.0 to 0.6° for x-ray reflectivity measurements, and the incident (I_0) and the reflectivity (I_1) x-rays were collected using Ar-filled ionization chambers. The fluorescence yield data were collected simultaneously using a 30-element Ge array detector. GI-XAFS spectra were collected at room temperature at an incidence angle of 0.10°. Pb and Zn metal foils were used during the experiments to calibrate energy. For GI-XAFS data analysis, a number of scans were averaged, background subtracted, and fit using the SIXPack interface (Webb, 2005).

2.7. LP-XSW-FY analysis

LP-XSW-FY data analysis includes three major steps. The first involves extracting the x-ray fluorescence intensity of each element from a full x-ray fluorescence spectrum measured with an energy dispersive detector. This is achieved using a peak fitting routine to produce background-removed fluorescence yield curves for each detected fluorescence peak. The second step is to fit the specular x-ray reflectivity using a routine based on Parratt's recursion formula (Parratt, 1954) with input of refractive indices from literature databases. The observed reflectivity data can be modeled to obtain the thickness, density,

and roughness of the film layer(s) with least squares fitting. The resulting fit parameters are used to calculate the XSW field intensity modulation, which is caused by the interference between the incident and reflected x-rays. XSW field intensity I_{xsw} , also known as electric field intensity, can be calculated from the complex amplitudes of the incident and reflected beams, respectively, using Equation 1 (Bedzyk and Cheng, 2002):

$$I_{\text{xsw}} = |E_i(z, \theta) + E_r(z, \theta)|^2 \quad [1]$$

where $E_i(z, \theta)$ and $E_r(z, \theta)$ are the complex amplitudes of the incident and reflected beams, both as a function of film depth, z , and x-ray incident angle, θ . This step is critical for carrying out subsequent quantitative LP-XSW-FY fitting. For the biofilm/metal-oxide/water systems studied here, the thickness of the film is too large to generate XSW field intensity modulations due to the limited coherence length of the incident and reflected beams. Therefore, the biofilm modulation was not included in reflectivity and electric field intensity calculations.

The electric field intensity modulation varies as a function of the depth and incident x-ray angle, and the emitted x-ray fluorescence intensity depends on the local electric field intensity. The fluorescence yield, $Y(\theta)$, can be modeled from the distribution function of the fluorescing species and the intensity modulation of the electric field intensity above the crystal surface using Equation 2 (Bedzyk and Cheng, 2002):

$$Y(\theta) \propto \int N(z) I_{\text{xsw}}(z, \theta) dz \quad [2]$$

where $N(z)$ is the distribution function for the metal ion and $I_{\text{xsw}}(z, \theta)$ is the XSW intensity. The final step of LP-XSW-FY data analysis is modeling the distribution function of the fluorescing species and combining the distribution function with the electric field intensity modulation. Although various distribution functions can be used to model the elemental distribution, a simple two-box model was used in this study because the film layer can be divided into two regions: one near the substrate surface with a thickness of 5 Å and another extending from 5 Å to the top of the biofilm. This simple distribution model assumes that metal ions associated with the biofilm accumulate in a single discrete, two-dimensional layer without vertical stratification. This two-box model is reasonable because, within a 5 Å region adjacent to the substrate surface, strong adsorption to the substrate surface is expected, whereas away from the surface the

distribution is not expected to change significantly, as suggested previously by Templeton et al. (2001). In this model, we used two free parameters representing the metal-ion concentrations within the two boxes. Attenuation of the emitted fluorescence intensity is accounted for in the LP-XSW-FY calculations. Finally, a least-square fitting routine was used to fit the parameters for the distribution model, from which the element partitioning profiles were obtained. The fitting routine was performed using custom-made XSW codes in the Igor program (Igor Pro, WaveMetrics, Lake Oswego, Oregon). The total fluorescent yield of the sample, which includes contributions from each box of the model, is roughly proportional to the total number of fluorescing atoms. The observed maximum variation in total number of Pb(II) and Zn(II) atoms is 16% (on average 7%) for different positions of the same sample or different samples measured under the same conditions. This error range is consistent with variations of biofilm thickness determined by CLSM, and it is expected as biofilms are inherently structurally heterogeneous, especially for the multilayer biofilms formed by *S. oneidensis* MR-1. The sensitivity of using a two-box model for metal-ion partitioning data was demonstrated by LP-XSW-FY simulations as described in detail in Appendix A2 and Fig. A5.

2.8 Langmuir model prediction of the metal-ion partitioning at biofilm/metal-oxide/water interfaces

Metal-ion partitioning at complex biofilm/metal-oxide interfaces can be described using a simple Langmuir model for the three component sorption systems (metal ions, biofilms, and metal-oxide surfaces) considered in this study:

$$\Gamma = \frac{nK_{app}[Me]}{1 + K_{app}[Me]} \quad [3]$$

where Γ is the equilibrium adsorption density (mol m^{-2}) for each type of adsorption site in the system, n is the site density of each adsorption site in the biofilm or on the metal-oxide surface, and K_{app} is the apparent binding constant for the metal ion associated with each reactive functional group or surface site. The adsorption density of each site can be calculated using the known site densities and the binding constants of various functional groups on the metal-oxide and bacterial cell wall surfaces. Templeton et al. (2001) used a multi-site Langmuir model (one biofilm site and two metal-oxide substrate sites) to fit

Pb(II) adsorption data at *B. cepacia* biofilm-coated α -Al₂O₃ (0001) and (1-102) and α -Fe₂O₃ (0001) single-crystal surfaces and obtained binding sites densities and Pb(II) binding affinities on these three surfaces. Following Templeton et al. (2001), two types of surface sites were fit: a strong binding site (edges, corners, kinks, and other defects on the surface) and a weak binding site (terrace site) on the three metal-oxide surfaces. By summing up the different reactive sites in the biofilms vs. the metal-oxide surfaces, Pb(II) adsorption in the biofilms vs. on the metal-oxide surfaces can be predicted using the following equation:

$$\frac{\Gamma_{biofilm}}{\Gamma_{total}} = \frac{\Gamma_{COOH} + \Gamma_{PO_4}}{\Gamma_{COOH} + \Gamma_{PO_4} + \Gamma_{M_{weak}} + \Gamma_{M_{strong}}} \quad [4]$$

where $\Gamma_{biofilm}$ is the fraction of Pb(II) sorbed in the biofilm, Γ_{total} represents the total density of adsorption sites in the system, Γ_{COOH} and Γ_{PO_4} are the fractions of Pb(II) sorbed to carboxyl and phosphoryl groups in the biofilm, respectively. $\Gamma_{Mstrong}$ and Γ_{Mweak} are the fractions of Pb(II) sorbed to strong and weak binding sites of the metal-oxide surface, respectively. The parameters used in model predictions are described in detail in Appendix A3.

3. RESULTS

3.1. Pb(II) distributions at *S. oneidensis* MR-1 biofilm/metal-oxide/water interfaces as a function of concentration after 3 hr of metal-ion exposure

Figures 4a-4c show the x-ray reflectivity and LP-XSW-FY profiles for Pb(II) partitioning at *S. oneidensis* MR-1 biofilm/metal-oxide/water interfaces as a function of Pb(II) concentration after 3 hr of metal-ion exposure. X-ray reflectivity curves for α -Fe₂O₃ (0001) surfaces indicate a critical angle (for total external reflection) of approximately $0.185^\circ \pm 0.010^\circ$ at 14 keV for the different α -Fe₂O₃ samples evaluated (Fig. 4a). The absence of fine structure modulations at incidence angles above the α -Fe₂O₃ critical angle is indicative of high biofilm-air interface roughness, which is

consistent with the CLSM results (Fig. 2). For α -Fe₂O₃ (0001) surfaces, and for the range of Pb(II) concentrations investigated, an evolution in the LP-XSW-FY profiles was observed as a function of incident x-ray angle (Fig. 4a). At low initial Pb(II) concentration (10⁻⁷ M), the LP-XSW-FY profile exhibits an intensity maximum close to the α -Fe₂O₃ critical angle. Increasing the initial Pb(II) concentration to 10⁻⁶ M results in two FY maxima with approximately equal intensities. The increase in intensity at lower angles (~0.1°) suggests that Pb(II) is partitioned more into the biofilm with increasing Pb(II) concentration after 3 hr of metal-ion exposure. For the higher initial Pb(II) concentrations (10⁻⁵ M and 10⁻⁴ M: 10⁻⁴ M data not shown), the low angle FY maximum dominates, which suggests that the biofilm matrix becomes the dominant sink for Pb(II) after 3 hr of metal-ion exposure.

Pb(II) partitioning at the biofilm-coated α -Al₂O₃ (1-102) surface as a function of metal-ion concentration (Fig. 4b) shows a trend similar to that observed for α -Fe₂O₃ (0001). The associated x-ray reflectivity curves indicate a critical angle of ~ 0.165° ± 0.010° at 14 keV, similar to the critical angle reported for the same oriented single-crystal type in earlier studies (Templeton et al., 2001; Yoon et al., 2005). Again, significant changes in the Pb LP-XSW-FY profiles occurred for the different Pb concentrations tested after 3 hr of metal exposure. Preferential partitioning to the substrate was observed at low initial Pb concentrations (10⁻⁷ M), whereas Pb partitioning dominantly into the biofilms was observed at high Pb concentrations (10⁻⁵ M).

The LP-XSW-FY profiles of Pb for the biofilm-coated α -Al₂O₃ (0001) surfaces (Fig. 4c) show a trend different from that of either the biofilm-coated α -Al₂O₃ (1-102) or α -Fe₂O₃ (0001) surfaces, with only one maximum occurring consistently at ~0.1° over the Pb concentration range tested, indicating that the biofilm is the dominant sink for Pb(II) under these conditions. This difference suggests that the reactivity of the biofilm-coated α -Al₂O₃ (0001) surface with respect to aqueous Pb(II) is lower than that of the other two surface types tested as discussed in more detail in Section 3.2.

Fitting of the LP-XSW-FY profiles using the two-box model allows extraction of quantitative information regarding the partitioning of Pb(II) between the biofilm coatings and the different metal-oxide surfaces. Table 1 presents the percentage of Pb(II) in the biofilms and on the metal-oxide surfaces for each metal-ion concentration examined. The

distributions of Pb(II) ions were averaged from measurements at different positions of the same sample, with each LP-XSW-FY replicate spectrum taken at a position at least 1 mm away from the other spectra, or from measurements on different samples of the same metal oxide with biofilm coating performed under the same conditions. The standard deviations are less than 10% for all samples.

As shown in Table 1, at an initial Pb(II) concentration of 10^{-7} M, ~60% of Pb(II) is partitioned to the α -Fe₂O₃ (0001) surface compared to ~40% into the biofilm. Similarly, in the case of α -Al₂O₃ (1-102) at low Pb concentration (10^{-7} M), Pb(II) is roughly equally partitioned between the metal-oxide surface (52%) and the biofilm (48%). In both cases, as the initial Pb(II) concentration increases, Pb(II) increasingly partitions into the biofilms. In contrast, fitting results indicate that in the case of biofilm-coated α -Al₂O₃ (0001) surfaces, partitioning of Pb(II) is dominantly into the biofilm (>99%) and thus there is no concentration dependence within the concentration range studied. In all cases, for the highest initial Pb(II) concentrations of 10^{-4} and 10^{-5} M, >99% of Pb(II) is associated with the biofilms, which suggests that the biofilms become the dominant sink for Pb(II) at high metal-ion concentrations for the three types of metal-oxide substrates and crystallographic orientations studied after 3 hr of metal-ion exposure. This LP-XSW-FY profile evolution suggests a higher affinity of Pb(II) for both α -Fe₂O₃ (0001) and α -Al₂O₃ (1-102) surface sites compared to the functional groups presents in the biofilm after 3 hr of metal-ion exposure.

3.2. Zn(II) distributions at *S. oneidensis* MR-1 biofilm/metal-oxide/water interfaces as a function of concentration after 3 hr of metal-ion exposure

Partitioning of Zn(II) for the three types of *S. oneidensis* biofilm-coated single-crystal surfaces as a function of Zn(II) concentration after 3 hr of metal-ion exposure was also evaluated using LP-XSW-FY spectroscopy (Figs. 5a-5c). The x-ray reflectivity curves for these samples are comparable to those of the Pb(II)-exposed samples described above. In general, the Zn(II) LP-XSW-FY profiles for α -Fe₂O₃(0001) show trends similar to those for Pb(II) on α -Fe₂O₃(0001). Table 2 presents the LP-XSW-FY modeling results for samples exposed to initial Zn concentrations ranging from 10^{-4} to 10^{-7} M. A significant amount of Zn(II) was sorbed onto α -Fe₂O₃(0001) at the lowest Zn concentration of 10^{-7}

M (45% at the surface compared to 55% in the biofilm). As Zn(II) concentration increased to 10^{-6} M, 59% of Zn(II) partitioned into the biofilm matrix and 41% partitioned to the metal-oxide surface. As the initial concentration of Zn(II) increased further to 10^{-5} M and 10^{-4} M, a maximum appears at $\sim 0.1^\circ$ in the Zn(II) LP-XSW-FY profile, indicating that >99% of Zn(II) partitioned into the biofilm.

Zn(II) partitioned more into the biofilms for the α -Al₂O₃(1-102) substrate relative to the Zn(II)/*S. oneidensis*/ α -Fe₂O₃(0001) system. For Zn(II) concentrations ranging from 10^{-4} to 10^{-6} M >99% of Zn(II) is found in the biofilms (Table 2). Even at the lowest Zn(II) concentration (10^{-7} M), most of the Zn(II) ($\sim 85\%$) is located in the biofilm matrix. However, an increase of the Zn LP-XSW-FY signal close to the critical angle of α -Al₂O₃(1-102) (0.165° at 14 keV) was also observed, which indicates that more Zn(II) partitions onto α -Al₂O₃(1-102) at low Zn(II) concentration ($\sim 15\%$) (Table 2). In contrast, the Zn(II) LP-XSW-FY profiles for the biofilm-coated α -Al₂O₃ (0001) surfaces show that the biofilms are the dominant sinks for Zn(II) (>99%) at all concentrations tested after 3 hr of metal-ion exposure (10^{-4} M to 10^{-7} M), which is similar to the results for the Pb(II)/*S. oneidensis*/ α -Al₂O₃(0001) system discussed above.

To compare the relative reactivities of the three substrates with respect to aqueous Pb(II) and Zn(II), the percentages of Pb(II) or Zn(II) in the biofilms for three types of metal-oxide surfaces are plotted in Fig. 6a and Fig. 6b, respectively. At the lower metal-ion concentrations (10^{-6} M and 10^{-7} M), the *S. oneidensis*/ α -Fe₂O₃(0001) system has a higher percentage of Pb(II) or Zn(II) (i.e. lower Pb(II) or Zn(II) uptake) at the substrate surfaces relative to the *S. oneidensis*/ α -Al₂O₃(1-102) and *S. oneidensis*/ α -Al₂O₃(0001) systems. These results indicate that the relative reactivities of the three single-crystal surfaces with respect to aqueous Pb(II) and Zn(II) follow the trend α -Fe₂O₃(0001) > α -Al₂O₃(1-102) $\gg$ α -Al₂O₃(0001).

3.3 Pb(II) and Zn(II) distributions at *S. oneidensis* MR-1 biofilm/metal-oxide/water interfaces as a function of metal-ion exposure time

Pb(II) and Zn(II) distributions at *S. oneidensis* MR-1 biofilm/metal-oxide/water interfaces were studied as a function of metal-ion exposure time for selected metal-oxide

surfaces and metal-ion concentrations. Fig. 7a shows x-ray reflectivity and LP-XSW-FY profiles for Pb(II) partitioning at *S. oneidensis* MR-1 biofilm-coated α -Fe₂O₃(0001) surfaces after 30 min, 3 hr, and 20 hr of metal exposure at an initial Pb(II) concentration of 10⁻⁵ M. Similar to the profiles of 3 hr metal-ion exposure samples described in Section 3.1, x-ray reflectivity profiles of 20 min and 20 hr samples show a critical angle of approximately 0.185° ± 0.010° at 14 keV and the absence of fine structure modulations (Fig. 7a). Note that the measured and fitted x-ray reflectivity profiles of the 30 min sample do not match at angles higher than the critical angle of α -Fe₂O₃ substrate. LP-XSW-FY data for this sample and several other samples collected at SSRL beamline 11-2 show similar problems in x-ray reflectivity data fitting. An inherent limitation of the spectrometer set-up at this beamline resulted in a high scattering background in x-ray reflectivity measurements. In this case, only qualitative information can be obtained from x-ray reflectivity and LP-XSW-FY data fitting.

An evolution in the LP-XSW-FY profiles was observed as a function of metal-ion exposure time for the Pb(II)/*S. oneidensis*/ α -Fe₂O₃(0001) system (Fig. 7a). At short exposure times (30 min and 3 hr), the LP-XSW-FY profiles exhibit intensity maxima in the low angle region ~0.1°, suggesting that most Pb(II) ions partition into the biofilms. After a longer exposure time (20 hr), Pb(II) FY intensity increases near the critical angle of α -Fe₂O₃ substrate, suggesting increased Pb(II) partitioning onto the α -Fe₂O₃(0001) surface. A similar trend was observed for Pb(II) partitioning in the *S. oneidensis*/ α -Al₂O₃(0001) system (Fig. 7b) at an initial Pb(II) concentration of 10⁻⁵ M. At short exposure times (30 min and 3 hr), the low angle FY maxima dominate in the LP-XSW-FY profiles, suggesting that the biofilms dominate the sorption of Pb(II) ions. After 24 hr of metal-ion exposure, Pb(II) FY intensity near the critical angle of α -Al₂O₃(0001) increases, indicating more Pb(II) ions partition onto α -Al₂O₃(0001) surface. Increased partitioning of Pb(II) to metal-oxide surfaces as a function of time was also observed in the Pb(II)/*S. oneidensis*/ α -Al₂O₃(1-102) system at concentrations of 10⁻⁵ (Fig. 8a) and 10⁻⁶ M (data not shown).

In the case of Zn(II), we performed one kinetic experiment on Zn(II) partitioning at *S. oneidensis*/ α -Al₂O₃(1-102)/water interfaces at an initial Zn(II) concentration of 10⁻⁵ M.

Similar to the case for Pb(II), an evolution in the LP-XSW-FY profiles was observed as a function of Zn(II) exposure time. At short exposure times (30 min and 3 hr), there is only one FY maximum in the low angle region ($\sim 0.1^\circ$) suggesting that biofilms dominate Zn(II) sorption. At a longer exposure time (24 hr), another FY maximum appears near the critical angle of α -Al₂O₃ substrate, indicating that more Zn(II) ions partition onto the α -Al₂O₃(1-102) surface. Table 3 lists the LP-XSW-FY fitting results for these samples. Note that several samples do not show a good match between LP-XSW-FY data and the fit, especially for samples that were reacted for longer times (20-24 hr). Relatively poor fits were also obtained for FY data collected on samples at SSRL beamline 11-2 where the x-ray reflectivity data have a high scattering background. Although the fitting results for these samples are only qualitative (Table 3), they consistently show a significant increase in the amount of metal ions onto the metal-oxide surfaces after longer metal exposure times for both Pb(II) and Zn(II) in the systems studied. For example, Pb(II) partitioning onto α -Fe₂O₃(0001) surfaces increases from 1% after 3 hr of reaction to $\sim 38\%$ after 20 hr of reaction at $[\text{Pb(II)}]_{\text{initial}} = 10^{-5}$ M; Pb(II) partitioning onto α -Al₂O₃(1-102) surfaces increases from $\sim 1\%$ after 3 hr of reaction to $\sim 46\%$ after 24 hr of reaction at $[\text{Pb(II)}]_{\text{initial}} = 10^{-5}$ M, and from $\sim 23\%$ after 3 hr of reaction to $\sim 61\%$ after 20 hr of reaction at $[\text{Pb(II)}]_{\text{initial}} = 10^{-6}$ M. Even for the least reactive surface, α -Al₂O₃(0001), we found that Pb(II) partitioning onto the α -Al₂O₃(0001) surface increases from 1% after 3 hr of reaction to $\sim 18\%$ after 24 hr of reaction at $[\text{Pb(II)}]_{\text{initial}} = 10^{-5}$ M. Similarly, Zn(II) partitioning onto α -Al₂O₃(1-102) surfaces increases from 1% after 3 hr of reaction to $\sim 25\%$ after 24 hr of reaction.

3.4. Pb L_{III}-Edge and Zn K-Edge GI-EXAFS results for the *S. oneidensis*/ α -Al₂O₃(1-102) system

Figures 9a and 9b present background-subtracted, normalized, and k^3 -weighted Pb(II) L_{III}-edge and Zn K-edge GI-EXAFS spectra and their corresponding Fourier transforms for the Pb(II)/*S. oneidensis* MR-1/ α -Al₂O₃(1-102) and Zn(II)/*S. oneidensis* MR-1/ α -Al₂O₃(1-102) systems, respectively, under *in situ* conditions at Me(II) concentrations of 10^{-5} M after 3 hr metal exposure. The spectra were collected in grazing-incidence mode at an incident angle of 0.100° , which is lower than the critical angle of α -Al₂O₃(1-102)

(0.180° at 13,055 eV for the Pb L_{III}-edge and 0.240° at 9,659 eV for the Zn K-edge). At these low angles, the GI-EXAFS measurements were sensitive primarily to Pb/Zn in the biofilms, providing unique information on local Pb/Zn bonding environments in the biofilms. Note that GI-XAFS measurements are difficult because they rely on precise goniometric control and are collected from a quasi-two-dimensional array of surface atoms, and, therefore, the resulting data may be limited in comparison to bulk EXAFS. The EXAFS fitting results (Table 4) indicate an average Pb-O distance of 2.38(±0.01) Å and an average first-neighbor coordination number (CN) of 2.0(±0.1) oxygens. The Pb-O bond length is consistent with those found in organic Pb compounds reported in the literature (2.25-2.8 Å) (Sarret et al., 1998; Templeton et al., 2003c).

Both carbon (carboxyl group) and phosphorus (phosphoryl group) were tested as second-shell neighbors around Pb. Adding the Pb-P(2nd-neighbor) pair correlation did not improve the fit, which suggests that phosphate may not be a significant ligand for Pb in this system. In contrast, addition of the Pb-C(2nd-neighbor) pair correlation improved the fit (χ^2 was reduced from 5.57 to 2.75) and resulted in an average Pb-C distance of 3.37(±0.03) Å and CN of 1.0(±0.3) carbons. The presence and bonding characteristics of the Pb-O and Pb-C(2nd-neighbor) shell together with the geometry constraints suggest that Pb(II) ions are likely to bind to oxygen in C-containing functional groups (e.g., carboxyl) in the bacterial cell walls and surface layers or the EPS, forming a monodentate inner-sphere complex.

To determine if the improvement in fit resulting from additional fitting parameters is statistically significant, we used a statistical F-test and the Nyquist criterion for the number of degrees of freedom to evaluate our fit results with and without a second-neighbor Pb-C shell (Downward et al., 2007). We found that using two-shell Pb-O + Pb-C contributions only marginally improved the fit at the 62.7% confidence level as compared to a one-shell contribution from Pb-O. Therefore, we could not definitively conclude that Pb(II) forms complexes with C-containing functional groups (e.g., carboxyl) in our systems, although that is a reasonable hypothesis given the biofilm chemistry.

Fitting of the normalized, background-subtracted, and k^3 -weighted Zn(II) K-edge GI-EXAFS spectrum for the Zn(II)/*S. oneidensis* MR-1/ α -Al₂O₃(1-102) system under *in situ*

conditions at a Zn concentration of 10^{-5} M indicates an average Zn-O distance of
 1.98(± 0.01) Å and an average first-neighbor coordination number (CN) of 3.6(± 0.4)
 oxygens (Table 4). The relatively short Zn-O distance is consistent with Zn(II) in a four-
 fold oxygen coordination environment. Again, both carboxyl and phosphoryl groups were
 tested as potential ligands around Zn(II). Addition of second-neighbor C resulted in an
 average Zn-C distance of 2.93(± 0.02) Å and second-neighbor CN of 2.2(± 0.6) carbons
 and improved the fit (χ^2 reduced from 14.51 to 8.75). These results suggest that Zn(II) is
 bonded to carboxyl groups in the biofilm. To test for the possibility of Zn binding to a
 phosphoryl group in the biofilm, we added a Zn-P(2nd-neighbor) pair correlation;
 however, the resulting Zn-P single scattering path does not match the 2nd neighbor peak
 position shown in the Fourier transforms (i.e. the Zn-P distance is unrealistic) (Fig. 9b),
 suggesting that phosphate may not be a significant ligand for Zn in the system tested.
 Although the Zn-C(2nd-neighbor) distance for Zn(II) in the biofilm (2.92 Å) is a bit
 higher than the 2.84 Å Zn-C distance reported for Zn(II) sorbed on free planktonic *S.*
oneidensis cells (Ha et al., 2010), it falls in the general range of Zn-C(2nd-neighbor)
 distances found in Zn-containing model organic compounds (2.6-3.6 Å) as well as Zn
 binding to other types of bacterial and fungal cell surfaces and humic materials (Sarret et
 al., 1998; Guine et al., 2006; Toner et al., 2005; Tourney et al., 2009). The difference in
 Zn-C distance found in this study compared to that reported in the study by Ha et al.
 (2010) may be due to different bonding environments resulting from different
 compositions of various C-containing functional groups between the planktonic cells
 studied by Ha et al. (2010) and the biofilm systems used in this study. The interatomic
 distances derived for the Zn-O and Zn-C(2nd-neighbor) and the bonding geometry
 constraints suggest that Zn(II) is likely bound to carboxyl groups as monodentate inner-
 sphere complexes.

However, our F-test results show that using both Zn-O and Zn-C shells vs. using only
 a single Zn-O shell improved the fit marginally at the 78.6% confidence level. Therefore,
 we cannot definitely conclude that Zn forms complexes with C-containing functional
 groups in our systems, although again, this is a reasonable hypothesis. Note that the
 XANES region of Zn(II) spectra (Appendix Fig. A6) show features similar to zinc acetate
 dehydrate reference spectra as well as zinc phosphate or zinc phytate spectra collected by

Lv et al., 2015. Therefore, we cannot conclude the presence of Zn(II) carboxylate complexes or Zn(II) phosphoryl complexes in the biofilms based on XANES either.

4. DISCUSSION

The partitioning of aqueous Pb(II) and Zn(II) cations between the *S. oneidensis* biofilm coating and the underlying metal-oxide substrates depends on competition for these cations by the various functional groups in the bacterial cell walls and EPS and the reactive sites on the metal-oxide surfaces. To provide a context for the results of the present study and a basis for developing a Langmuir model for Pb(II) and Zn(II) sorption on the different components of the biofilm/metal-oxide system, we present a brief summary of recent studies of the types and properties of functional groups in bacterial cell walls and biofilm EPS as well as the results of selected studies of metal-ion uptake by cell-wall and biofilm functional groups. We also discuss the types and affinities of reactive sites on Fe- and Al-oxides, which compete for aqueous metal ions with the biofilm functional groups and conclude with a discussion of the implications of our findings regarding the effects of biofilm coatings on metal oxide reactivity with aqueous Pb(II) and Zn(II).

4.1. Metal-ion adsorption properties of bacterial cell walls and biofilm matrix

Bacterial surfaces contain a high abundance of various functional groups including carboxyl, phosphoryl, hydroxyl, amine, and sulfhydryl groups (e.g., Fein et al., 1997; Wei et al., 2004). There have been extensive studies of proton and metal-ion adsorption onto planktonic bacterial cells (e.g., Fein et al., 1997; Ginn and Fein, 2008 and references therein). Several studies have shown that various strains of bacteria, either pure or mixed bacterial consortia, lab cultured or isolated from natural environments, exhibit similar proton and metal cation adsorption behavior, i.e. comparable protonation constants, site densities, and Cd(II) binding constants for carboxyl, phosphoryl, hydroxyl, and amine groups (Yee and Fein, 2001; Borrok et al., 2005; Johnson et al., 2007; Ginn and Fein, 2008). Based on these observations, a universal proton and metal-ion adsorption model for metal-ion/bacteria interactions was proposed that simplifies the

incorporation of bacterial adsorption of metal cations into general geochemical speciation models (Yee and Fein, 2001; Borrok et al., 2005; Johnson et al., 2007; Ginn and Fein, 2008).

Application of this universal model can be challenging, however, due to the enormous diversity in the microbial world and the large uncertainties associated with experimental protocols and different modeling approaches used in various studies. In addition, comparison of metal-ion binding affinities for different strains of bacteria is not straightforward because most of the studies do not have data for the same metal ions and some of the binding affinities obtained are not well constrained (Yee and Fein, 2001; Fein et al., 2005). There are large ranges of site densities and binding affinities found for various bacterial strains from different studies as shown in Fig. 10 and Table 5, which may reflect differences in experimental protocols, such as different growth media, solid phases, and ionic strengths, and various modeling approaches used in these studies.

Although the phosphoryl group densities of planktonic *S. oneidensis* MR-1 cells found by Ha et al. (2010) are comparable to the average values of the natural and contaminated bacterial consortia found by Ginn and Fein (2008), the carboxyl group densities are about 50% higher (Table 5). Several studies have shown that Pb(II) and Zn(II) complex with phosphoryl groups much more strongly than with carboxyl groups (1-2 log units higher) for planktonic cells, as shown in Table 5 (Fein et al., 1997; Daughney et al., 1998; Guine et al., 2006; Ngwenya et al., 2003). In contrast, Ha et al. (2010) found that Zn(II) ions have comparable binding constants to phosphoryl and carboxyl groups in *S. oneidensis* planktonic cells. In addition, Ha et al. (2010) derived a Pb(II) binding affinity log K value with *S. oneidensis* MR-1 cells that is 2.4 log units lower than that found by Ginn and Fein (2008) from the averaged log K values of five different bacterial species. Because there have been no potentiometric titrations or metal ion sorption experiments performed on *S. oneidensis* MR-1 biofilm cells, the site densities and metal binding affinities from the study by Ha et al. (2010) were used by us in a Langmuir model analysis (see section 4.3).

EPS associated with bacterial cells and biofilms also plays an important role in proton and metal-ion adsorption properties of planktonic bacteria and biofilms (Comte et al., 2006; Boonfueng et al., 2009; Ha et al., 2010). However, the composition of EPS is

believed to vary widely with growth conditions, including the type of medium, growth time, temperature, and oxygen content (Costerton et al., 1995; Donlan and Costerton, 2002). Some studies have suggested that EPS could contribute significant amounts of phosphoryl groups (Guine et al., 2006), whereas others have shown that EPS also contributes considerable amounts of carboxyl groups (Boonfueng et al., 2009; Ha et al., 2010). Ha et al. (2010) studied Pb(II) and Zn(II) adsorption on *S. oneidensis* MR-1 wild type cells and on a genetically modified EPS mutant strain in which EPS production was inhibited. Decreased concentrations of carboxyl (~48% reduction) and amide groups but increased concentrations of phosphoryl groups were found in EPS-deficient cells, which resulted in much lower uptake of both Pb(II) and Zn(II) ions. They also found that Zn(II) dominantly formed Zn carboxyl complexes on *S. oneidensis* wild type cells at pH 4, with a possible increasing contribution of Zn phosphoryl complexes at pH 7 (Ha et al., 2010). The presence of Zn phosphoryl complexes at pH 7 was found to be more pronounced in EPS-deficient mutant cells, suggesting that removal of EPS from the cells allowed more Zn(II) ions to diffuse into the cell walls and form Zn phosphoryl complexes (Ha et al., 2010). Thus, it is possible that the presence of EPS may inhibit Pb(II) or Zn(II) ions from diffusing close to the bacterial cell walls in the case of biofilms.

It is expected that larger amounts of EPS are present in the *S. oneidensis* biofilms in the present study compared to the planktonic *S. oneidensis* cells grown in the rich medium used by Ha et al. (2010), and thus more carboxyl groups would be present than phosphoryl groups in the biofilms. Based on the site densities and binding affinities shown in Table 5, carboxyl functional groups are expected to dominate Pb(II) and/or Zn(II) adsorption on the *S. oneidensis* MR-1 biofilms at pH 6. This suggestion is consistent with the findings from our GI-EXAFS measurements, which suggest (but don't prove) that Pb(II) and Zn(II) ions are likely to complex with carboxyl groups in the *S. oneidensis* biofilms. The statistical certainty of this hypothesis is not as robust as generally found for conventional EXAFS analysis because of the limited GI-EXAFS data range, beam instabilities, background problems, and the fact that changing x-ray energy during the GI-EXAFS scans changed the critical angle of the metal oxide substrate (see Section 3.4).

4.2. Metal-ion adsorption properties of α -Al₂O₃ and α -Fe₂O₃ single-crystal surfaces

Pb(II) and Zn(II) adsorption on metal-oxide surfaces has been extensively studied in the past. Spectroscopic techniques such as EXAFS have been widely used since the 1990s to study the nature and local structural environment of adsorbed species on metal-oxide surfaces (Brown and Sturchio, 2002) and can be used to validate various surface complexation models (Bradl, 2004). For example, there have been several GI-EXAFS studies of Pb(II) and Zn(II) adsorption onto α -Al₂O₃ and α -Fe₂O₃ oriented single-crystal surfaces (Bargar et al., 1996; Trainor et al., 2001; Bargar et al., 2004). Bargar et al. (1996) found that the mode of Pb adsorption on α -Al₂O₃ is different on the (0001) and (1-102) hydrated single-crystal surfaces, with Pb(II) forming dominantly outer-sphere adsorption complexes on the hydrated (0001) surface and dominantly inner-sphere adsorption complexes on the hydrated (1-102) surface. In contrast, dominantly inner-sphere Pb(II) adsorption complexes were found on both the hydrated single-crystal α -Fe₂O₃ (0001) and (1-102) surfaces (Bargar et al., 2004). These studies suggest that Pb(II) and Zn(II) can form strong complexes with reactive surfaces such as α -Al₂O₃ (1-102) and α -Fe₂O₃ (0001), and thus, in principle, can strongly compete with biofilms for metal cation adsorption.

In general, the trend in affinity of Pb(II) for the three metal-oxide surfaces considered here (without biofilm coating) is: α -Fe₂O₃(0001) > α -Al₂O₃ (1-102) >> α -Al₂O₃ (0001) (Templeton et al., 2001). These findings are in agreement with the differences in the relative reactivities reported here for the three substrates coated with a multi-layer *S. oneidensis* biofilm (see Sections 3.3 and 3.4). This result is also consistent with the trend observed for Pb(II) partitioning in *B. cepacia* monolayer biofilm-coated metal-oxide surfaces (Templeton et al., 2001) and Pb(II), Zn(II), and As(V) partitioning in polyacrylic acid polymer film-coated metal-oxide surfaces (Yoon et al., 2005; Wang et al., 2013). These differences in reactivity among three oriented metal-oxide surfaces are related to the surface structure of the terminating plane (Brown et al., 2007) as well as to the differences in electronic structure (Mason et al., 2009; Mason et al., 2010; Aboud et al., 2011; Mason et al., 2011). They are also related to the differences in the dissociative chemisorption of water on these surfaces. For example, Liu et al. (1998) found that the threshold water vapor pressure for the dissociative chemisorption of water vapor on α -

Fe₂O₃ (0001) surfaces is 10⁻⁴ Torr, whereas the threshold pressure for dissociative chemisorption of water on the α -Al₂O₃ (0001) surface is ~ 1 Torr (Liu et al., 1998). An *ab initio* thermodynamic-DFT investigation of Pb(II) adsorption on hydrated α -Al₂O₃ (0001) and α -Fe₂O₃ (0001) surfaces showed that the adsorption energy of Pb(II) on α -Fe₂O₃ (0001) is more than four times that of its α -Al₂O₃ (0001) counterpart (Mason et al., 2009).

In comparison, Trainor et al. (2001) found that Zn(II) binds to α -Al₂O₃ (0001) and (1-102) single-crystal surfaces as dominantly inner-sphere adsorption complexes with Zn-O distances ranging from 1.97 to 1.99 Å, indicating that Zn(II) is coordinated to four oxygens. Zn(II) adsorption on single-crystal α -Fe₂O₃ (0001) has not been studied, to the best of our knowledge. In addition, there are no potentiometric titration and metal-ion adsorption studies coupled with surface complexation modelling on α -Al₂O₃ (0001) and (1-102) or on α -Fe₂O₃ (0001) single-crystal surfaces

It is worth noting that single-crystal metal-oxide surfaces, such as those used in the present study, have isoelectric points (IEPs) that are typically several pH units lower (pH of 4.5 to 6.5) than their powdered polycrystalline counterparts (pH of 8-9) (Franks and Gan, 2007; Wang et al., to be submitted). This difference has been attributed to a higher concentration of defect sites on the particle surfaces relative to the single-crystal surfaces (Franks and Gan, 2007; Wang et al., to be submitted). As a result, the single-crystal surfaces should be slightly negatively charged or neutral, whereas α -Al₂O₃ and α -Fe₂O₃ surfaces in polycrystalline samples should be positively charged at the pH (6.0) of our experiments. These differences should result in less electrostatic repulsion of aqueous Pb(II) and Zn(II) as these ions approach the single-crystal metal-oxide surfaces relative to the particle surfaces. The effect of pH on Pb(II) partitioning between the *S. oneidensis* biofilm and the α -Al₂O₃(1-102) surface is illustrated in Fig. 11, which shows that Pb(II) is more strongly partitioned into the *S. oneidensis* biofilm coating at pH 4 (more FY intensity at 0.1° than at 0.165°) than at pH 6 (roughly equal FY intensities at the two incident angles). Because the IEP of α -Al₂O₃(1-102) is 5.1 (Wang et al., to be submitted), the alumina surface should be slightly positively charged at pH 4 and slightly negatively charged at pH 6, thus favoring more enhanced adsorption of Pb(II) on the alumina

surface at the higher pH value, as observed. As micro- and nano-particles of metal oxides are more common in natural environments than single-crystal substrates, adsorption of divalent metal ions such as Pb(II) and Zn(II) on small metal-oxide particles must overcome the electrostatic repulsion between the aqueous cations and the positively charged metal-oxide particle surfaces at near-neutral pH values by forming direct chemical bonds to surface sites. There is strong evidence from many adsorption isotherm and EXAFS studies of adsorption products on metal oxide surfaces that this is indeed the case (e.g., Brown and Sturchio, 2002).

4.3. Model predictions of Pb(II) distributions at biofilm/metal-oxide interfaces

The percentages of metal ions in the biofilm and on the metal-oxide surfaces as a function of aqueous Pb(II) concentration after 3 hr of metal-ion exposure were predicted using the Langmuir model and are summarized in Table 1. The Langmuir model we employed was described in Section 2.7 and the parameters used in this model are listed in Table 6. Table 1 show that as Pb(II) concentration is increased, more Pb(II) will partition into the biofilm for the α -Al₂O₃ (1-102) and α -Fe₂O₃ (0001) substrates. For the relatively unreactive α -Al₂O₃ (0001) substrate, the model predicts that biofilms are the dominant sinks for Pb(II). These trends agree well with the measured LP-XSW-FY data, which are also listed in Table 1. However, the Langmuir-predicted values for Pb(II) partitioning on α -Al₂O₃ (1-102) and α -Fe₂O₃ (0001) surfaces are generally lower than the LP-XSW-FY-measured partitioning values for the lower Pb(II) concentrations ($\leq 10^{-6}$ M). For example, at a Pb(II) concentration of 10^{-6} M, the Langmuir model predicts that 11% of Pb(II) partitioned to the α -Fe₂O₃ (0001) surface, whereas 26% was derived from the LP-XSW-FY measurements. The discrepancy becomes larger as the concentration is lowered to 10^{-7} M; 30% of Pb(II) is predicted to partition to the α -Fe₂O₃ (0001) surface using the simple Langmuir model, whereas ~60% of the Pb(II) was found to partition to the α -Fe₂O₃ (0001) surface from the LP-XSW-FY measurements. Considering the uncertainty of the values of site densities and binding constants of the biofilm functional groups and mineral surface binding sites used in the Langmuir model predictions and the estimated errors of the LP-XSW-FY experimental data (<10%), these predictions are within the range of uncertainty associated with the model predictions. As the site densities and

binding constants were taken from planktonic cells of *S. oneidensis* MR-1 grown in very rich media compared to the biofilms that we grew in the present study under nutrient-limited conditions, these differences could lead to an overestimation of biofilm adsorption using the additive Langmuir model.

It is also possible that the binding affinities of Pb(II) for the strong and weak binding sites on the metal-oxide surfaces and the metal-oxide surface site densities were underestimated by Templeton et al. (2001). For example, recent crystal truncation rod x-ray diffraction studies have revealed three types of functional groups, including singly coordinated MO, doubly coordinated M_2O , and triply coordinated M_3O , equally present on the $\alpha\text{-Al}_2\text{O}_3$ (1-102) and $\alpha\text{-Fe}_2\text{O}_3$ (0001) surfaces, whereas doubly coordinated M_2O groups are dominantly present on the inert $\alpha\text{-Al}_2\text{O}_3$ (0001) surface, where $M = \text{Al}$ or Fe^{3+} (Eng et al., 2000; Trainor et al., 2002; Trainor et al., 2004). Considering that singly coordinated MO groups are the most reactive and strongest binding sites on the metal-oxide surfaces examined here, using a strong site to weak site ratio of 1:10, as assumed by Templeton et al. (2001), leads to an underestimation of the percentage of strong binding sites for the $\alpha\text{-Al}_2\text{O}_3$ (1-102) and $\alpha\text{-Fe}_2\text{O}_3$ (0001) surfaces. In contrast, this assumption leads to an overestimation of adsorption of Pb(II) ions on the inert $\alpha\text{-Al}_2\text{O}_3$ (0001) surface, where unreactive M_2O sites are dominant. This reasoning is consistent with the observed differences in percentage partitioning values of Pb(II) derived from a Langmuir model vs. those from the LP-XSW-FY measurements (Table 1). Assuming singly coordinated MO groups as strong binding sites, and doubly coordinated M_2O and triply coordinated M_3O groups as weak binding sites on the metal-oxide surfaces, new site densities were calculated and are listed in Table 6. Using these site densities and the corresponding Pb(II) binding affinities from Templeton et al. (2001), the calculated percentages of Pb(II) adsorbed on the metal-oxide surfaces are much closer to the values obtained by LP-XSW-FY measurements in the present study (see Table 1).

Mason et al. (2009) used DFT calculations to study Pb(II) adsorption on hydrated $\alpha\text{-Al}_2\text{O}_3$ (0001) and $\alpha\text{-Fe}_2\text{O}_3$ (0001) surfaces and found that the predicted adsorption energy of Pb(II) on $\alpha\text{-Fe}_2\text{O}_3$ (0001) is more than four times greater than that of Pb(II) on $\alpha\text{-Al}_2\text{O}_3$ (0001) (Mason et al., 2009). This prediction indicates that the $\log K_{app}$ values for Pb(II) adsorption on the $\alpha\text{-Fe}_2\text{O}_3$ (0001) surface should be at least 1.74 log units (55

times) higher than that of the α -Al₂O₃ (0001) surface (Mason et al., 2009). However, the log K_{app} value for the strong sites on the α -Fe₂O₃ (0001) surface (in Table 6) is 1.3 log units (20 times) higher than α -Al₂O₃ (0001), whereas the log K value for the weak sites is only 0.8 log units (6 times) higher, indicating either an overestimation of reactive sites on α -Al₂O₃ (0001) or an underestimation of reactive sites on α -Fe₂O₃ (0001).

The differences between the Langmuir model partitioning percentages relative to the values obtained from our LP-XSW-FY measurements may also be related to the local microenvironments that can be created by bacterial metabolic activity or mass transfer limitations in the biofilm system, as the Langmuir model assumes chemical equilibrium, which is not likely to be true for the relatively short reaction times used in the present and past studies of metal-ion partitioning at biofilm/metal-oxide interfaces (see section 4.4). We also used the Langmuir model to predict Pb(II) distributions at biofilm/metal-oxide/water interfaces for the longer exposure time samples and found significant discrepancies between the experimental data and the model predictions as shown in Table 3. It is possible that bacteria create microenvironments which induce metal ions to form precipitates after longer exposure times at biofilm/metal-oxide/water interfaces as mentioned in section 3.3. Another possible explanation for these differences is that bacteria can induce the formation of organic polymers on the metal-oxide surfaces, which promote formation of strong ternary complexes at the interfaces and thus enhance the adsorption of Pb(II) ions on the metal-oxide surfaces. Similar predictions cannot be made for Zn(II) because of the lack of thermodynamic data of Zn(II) interaction with various surfaces.

4.4. Kinetics of Pb(II) and Zn(II) sorption reactions at biofilm-metal oxide interfaces

Based on the results presented in section 3.3, a general trend for Pb(II) and Zn(II) partitioning at biofilm/metal-oxide/water interfaces is likely to occur as a function of metal-ion exposure time: at short exposure times (20-30 min), the biofilms are the major sink for metal ion sorption, whereas at longer exposure times (20-24 hr), Pb(II) and Zn(II) increasingly partition onto the metal-oxide surfaces, indicating continuing diffusion of these metal ions through the biofilm coatings to the metal-oxide substrates over longer exposure times. These findings suggest that the reactive sites on the

underlying metal-oxide surfaces may not be completely filled by metal ions in exposures of 20 to 30 minutes or even 3 hours, which was the reaction time used in most of the present study as well as in the earlier study of Pb(II) partitioning between *B. cepacia* biofilms and the underlying metal-oxide substrates by Templeton et al. (2001). The present study is the first to our knowledge that shows the kinetic effect of metal-ion sorption at complex biofilm/metal-oxide/water interfaces. Increased Pb(II) and Zn(II) partitioning onto metal-oxide surfaces with time demonstrates that sorption is diffusion-limited at the biofilm/mineral/water interface. Although the biofilms do not block reactive sites on the metal-oxide surfaces as shown in the 3 hr metal-ion exposure data described in Section 3.1 and 3.2, the kinetic study shows that biofilms reduce the rate of metal-ion sorption onto the underlying surfaces. Chen and Stewart (1996) found in their system that reaction and diffusion interaction between chlorine and biofilms significantly delayed the transport of chlorine in the biofilms (a time lag of 144 hours for a reaction/diffusion mechanism in contrast to a time lag of 44 seconds for diffusion only). The observed increased partitioning of metal ions to the underlying metal-oxide surfaces at longer exposure times suggests a similar mechanism in our system that metal ions are likely to complex/react with the biofilms, which causes a delay of metal ions in reaching the metal-oxide surfaces. Chen and Stewart's results indicate a possible diffusion/reaction controlled sorption process occurring at biofilm/metal-oxide/water interfaces.

Note that the fitting results of LP-XSW-FY data presented in Table 3, especially for longer exposure times, can only be used qualitatively because of the noticeable discrepancies between data and fits. The LP-XSW-FY data often show a high background in the high angle region and are not well fit with the two-box model. We suggest that these poor fits may be due to the formation of solid precipitates at biofilm/metal-oxide/water interfaces after longer exposures. However, analysis of our GI-XAFS data for Pb(II) and Zn(II) in the biofilms or at the interfaces after longer exposure times did not reveal such precipitates for the reasons discussed in section 4.1 as well as below.

The metal/biofilm/metal-oxide/water interfaces studied here are complex and dynamic with respect to reaction time because the biofilm coatings were degrading over long reaction times due to toxic metal stress and starving conditions (an 0.01 M NaNO₃

solution was used as a simple background electrolyte without nutrient medium to avoid possible metal hydroxide/carbonate/phosphate precipitation). We observed about 30% loss of biomass in biofilms after 24 hour of reaction with 10^{-5} M Pb(II) in *S. oneidensis* MR-1/ α -Al₂O₃ (1-102)/water interfaces. In addition, it is possible that biofilm metabolic states were quite different after longer reaction times with toxic metal stress and starving conditions. So it is difficult to compare the partitioning data at different reaction times because the system is changing over time. True equilibrium conditions cannot be reached in such dynamic systems, which presents a significant challenge for evaluating kinetic effects.

4.5. Implications

The observed partitioning behavior of aqueous Pb(II) and Zn(II) at the biofilm-coated metal-oxide surfaces examined in this study for 3-hr exposures to these metal ions can be explained by considering the site densities and binding affinities of various functional groups in the biofilms and reactive sites on the metal-oxide surfaces. As shown in Table 6, all reactive functional groups associated with the biofilms have weaker binding affinities with respect to Pb(II) compared to the strongest but less abundant binding sites (singly coordinated MO sites) of the reactive surfaces such as α -Al₂O₃ (1-102) and α -Fe₂O₃ (0001), whereas the two weaker binding sites (M₂O and M₃O sites) of α -Al₂O₃ (1-102) and α -Fe₂O₃ (0001) should have lower binding affinities than biofilm carboxyl and phosphoryl groups. These two weaker binding sites, however, represent a substantially larger fraction of the total reactive sites (2 times higher) on the single-crystal surfaces compared to the strongest binding MO site. Therefore, as shown schematically in Fig. 12 at lower Pb(II) concentrations, aqueous Pb(II) preferentially binds to the strongest binding MO sites on the α -Al₂O₃ (1-102) and α -Fe₂O₃ (0001) surfaces. As these sites become increasingly occupied, the carboxyl and phosphoryl groups in the biofilms should become dominant adsorption sites for Pb(II) in terms of binding affinities and site densities. The weaker binding sites on the metal-oxide surfaces are expected to be the least favorable sorption sites for Pb(II), based on the binding affinity values shown in Table 6. In contrast, the binding constants of weaker binding sites (M₂O, about 95% of the total sites (Liu et al., 1998)) on the less reactive α -Al₂O₃ (0001) surfaces are lower

than those of both the carboxyl and phosphoryl groups in the biofilms, whereas the stronger binding defect sites of this surface are much less populated (about 5% (Liu et al., 1998)) and they exhibit binding affinities closer to the carboxyl and phosphoryl values. Therefore, the biofilms become dominant sinks for Pb(II) at all the concentrations examined for the biofilm-coated α -Al₂O₃ (0001) surfaces.

This analysis indicates that the binding affinities and site densities of various reactive functional groups in the biofilms and on the metal-oxide surfaces are key factors controlling the types of Pb(II) surface complexes formed under the fixed experimental conditions of our study, including pH and ionic strength. Templeton et al. (2001) showed similar trends for aqueous Pb(II) adsorption at *B. cepacia* monolayer biofilm-coated metal-oxide surfaces with Pb(II) partitioned mostly to the reactive metal-oxide surfaces at low concentrations, whereas the biofilms became the dominant sinks for Pb(II) adsorption at high Pb(II) concentrations. Adsorption of Pb(II) to *B. cepacia* biofilm-coated goethite (α -FeOOH) particles showed similar trends (Templeton et al., 2003a), where Pb(II) at low concentrations adsorbed primarily onto goethite particles; in contrast, the biofilm contributed significant adsorption sites at higher Pb(II) concentrations. Similarly, Toner et al. (2006) studied Zn(II) adsorption onto a *Pseudomonas putida* biofilm-embedded biogenic Mn-oxide and found that Zn(II) was mostly adsorbed to reactive sites on Mn-oxides at low Zn(II) loadings and dominantly to biofilm sites only after most of the reactive sites on the Mn-oxides had become occupied. Dong et al. (2000) used several selective chemical extraction techniques to study Pb(II) and Cd(II) adsorption onto biofilms and associated minerals collected from surface waters of Cayuga Lake, New York. They found that adsorption of Cd(II) is mostly dominated by Fe-oxides compared to Mn- and Al-oxides and by the organic materials associated with the biofilms, whereas adsorption of Pb(II) is mostly dominated by Mn-oxides followed by Fe-oxides, biofilms and Al-oxides (Dong et al., 2000). This result is expected considering the relatively high reactivities of Mn- and Fe-oxides compared to the biofilms. The results of our study of model systems of *S. oneidensis* biofilms grown on single-crystal metal-oxide surfaces are in good agreement with what Dong et al. (2000) found for natural samples. In natural systems, minerals are mostly present in particulate or nanoparticulate forms, which are expected to be much more reactive compared with

single-crystal metal-oxide surfaces due to the presence of various defects and high surface areas. Based on the results of the present study, we can predict that metal-ion partitioning in complex biofilm/nanoparticulate mineral systems is likely to be controlled by reactive mineral surfaces at low metal ion concentrations.

The strong adsorption sites often present at low densities on metal-oxide surfaces are difficult to identify and can be masked, particularly when the experimental conditions typical of most studies involve the use of relatively high metal-ion concentrations. EXAFS studies of metal-ion adsorption on bacterial cell wall surfaces are good examples. For most of the studies reported in the literature, the types of metal-ion binding sites observed in metal-ion/bacteria adsorption studies are mostly carboxyl and phosphoryl groups (e.g., Guine et al., 2006; Toner et al., 2005; Ha et al., 2010). Highly reactive functional groups in bacterial cell walls such as sulfhydryl groups have only been detected at very low metal-ion loadings (Guine et al., 2006; Mishra et al., 2009). Data at much lower metal ion to surface site concentration ratios are needed to probe the high affinity sites of these cell walls. For lower metal-ion concentrations, which are in general more environmentally relevant, the LP-XSW-FY method has excellent sensitivity for probing the few strong adsorption sites.

Biofilms are well known to have functional, structural, and chemical heterogeneities. The Pb(II) binding affinities and site densities of carboxyl and phosphoryl groups in Table 4 were derived from thermodynamic modelling of adsorption on planktonic cells without a mass transfer limitation. As described in the introduction, one major difference between planktonic cells and biofilm cells is the extensive polymer network (i.e. EPS) associated with the biofilms, which could hinder the transport of metal ions from biofilms to metal-oxide surfaces. In this study, an evolution of the LP-XSW-FY partitioning profiles was observed as a function of time for both Pb(II) and Zn(II) at 10^{-5} M: at short exposure times (20-30 minutes) the biofilm was found to be the major sink for metal ion sorption, whereas at longer exposure times (24 hours) Pb(II) and Zn(II) were found to increasingly partition onto the mineral surface, indicating continuing diffusion of these ions through the biofilm coating to the metal-oxide substrates. These findings also indicate that the reactive sites on the underlying metal-oxide surfaces are not completely filled by metal ions in exposures of 20 to 30 minutes or even 3 hours, which was the

maximum reaction time used in the present study as well as in the earlier study of Pb(II) partitioning between *B. cepacia* biofilms and the underlying metal-oxide substrates by Templeton et al. (2001). This evolution in the LP-XSW-FY profiles with time demonstrates that diffusion-limited sorption processes occur at the biofilm/mineral interface.

5. CONCLUSIONS

This study confirms the general trend observed for metal-ion adsorption on biofilm-coated Al- and Fe-oxide surfaces by Templeton et al. (2001); however, a more environmentally realistic multilayer *S. oneidensis* MR-1 biofilm was used in the present study. We conclude that such biofilms do not block reactive sites on metal-oxide surfaces but instead provide additional adsorption sites and reduce the rate of metal-ion sorption onto underlying metal-oxide surfaces. The relative order of reactivity of hydrated metal-oxide surfaces to aqueous Pb(II) and Zn(II) ions [α -Fe₂O₃(0001) > α -Al₂O₃ (1-102) >> α -Al₂O₃ (0001)] is preserved even when coated by thick *S. oneidensis* biofilms after 3 hr of metal-ion exposure. In natural environments, metal ions are often present at relatively low concentrations ($< 10^{-7}$ M), thus reactive metal-oxide surfaces such as those on Fe- and Al-oxides should dominate the adsorption of metal ions. At elevated metal-ion concentrations, such as at contaminated sites, the presence of biofilms may play an important role in governing the fate and transport of metal ions by providing a significant concentration of additional adsorption sites. In addition, we found that reaction times longer than 3 hours resulted in increasing partitioning of Pb(II) and Zn(II) from the overlying *Shewanella oneidensis* biofilms to the metal-oxide substrates, suggesting that the biofilm reduced the rate of metal-ion sorption on the metal-oxide substrates. This is the first study to show evidence of a diffusion-controlled metal-ion sorption process occurring at complex biofilm/metal-oxide/water interfaces. The results of this study provide new insights about how microbial biofilm coatings affect mineral reactivity and metal-ion sorption behavior and thus improve our understanding of metal-ion partitioning at complex biofilm/metal-oxide/water interfaces. They also have important implications for designing efficient bioremediation strategies for cleaning up heavy-metal pollutants.

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Fig. 1. Schematic representation of experimental set-up for biofilm growth.

Fig. 2. Confocal laser scanning microscopy (CLSM) images of green fluorescent protein (*gfp*)-labeled *S. oneidensis* MR-1 biofilms grown on $\alpha\text{-Fe}_2\text{O}_3(0001)$, $\alpha\text{-Al}_2\text{O}_3(1-102)$, and $\alpha\text{-Al}_2\text{O}_3(0001)$ surfaces after 10 days. The average biofilm thickness for $\alpha\text{-Fe}_2\text{O}_3(0001)$, $\alpha\text{-Al}_2\text{O}_3(1-102)$, and $\alpha\text{-Al}_2\text{O}_3(0001)$ surfaces are 20 μm , 9 μm , and 6 μm , respectively. The bottom and right-hand side panels are the x-z and y-z cross-section images at selected positions in the biofilm, respectively.

Fig. 3. Schematic representation of x-ray reflectivity, LP-XSW-FY, and GI-XAFS configuration.

Fig. 4. Observed x-ray reflectivity profiles (open grey circles) and LP-XSW Pb L α fluorescence yield (FY) profiles (open red diamonds), with calculated fits overlain (grey and red solid lines, respectively). Evolution of LP-XSW-FY profiles with increasing initial Pb(II) concentration indicate a change in the distribution of Pb(II) between the *S. oneidensis* MR-1 biofilm matrix and the metal-oxide substrates: (a) α -Fe $_2$ O $_3$ (0001) surfaces, (b) α -Al $_2$ O $_3$ (1-102) surfaces, and (c) α -Al $_2$ O $_3$ (0001) surfaces. Samples with initial Pb(II) concentrations of 10^{-4} M were also measured for α -Fe $_2$ O $_3$ (0001) and α -Al $_2$ O $_3$ (1-102), but data are not shown (see Table 1 for fit results).

Fig. 5. Observed x-ray reflectivity profiles (open grey circles) and LP-XSW Zn K α fluorescence yield (FY) profiles (open blue diamonds), with calculated fits overlain (grey and blue solid lines, respectively). Evolution of LP-XSW-FY profiles with increasing initial Zn(II) concentration indicate changes in the distribution of Zn(II) between the *S. oneidensis* MR-1 biofilm matrix and the metal-oxide substrates: (a) α -Fe $_2$ O $_3$ (0001) surfaces, (b) α -Al $_2$ O $_3$ (1-102) surfaces, and (c) α -Al $_2$ O $_3$ (0001) surfaces. Samples with initial Zn(II) concentrations of 10^{-4} M were also measured for α -Fe $_2$ O $_3$ (0001) and α -Al $_2$ O $_3$ (1-102), but data are not shown (see Table 2 for fit results).

Fig. 6. The percentage metal-ion distributions in the biofilms for *S. oneidensis* MR-1 biofilm coated mineral surfaces, pH = 6.0, exposure time = 3 hrs: (a) Pb(II), and (b) Zn(II).

Fig. 7. Pb(II) LP-XSW-FY profiles on *S. oneidensis* MR-1 biofilm-coated $\alpha\text{-Fe}_2\text{O}_3$ (0001) (a) and $\alpha\text{-Al}_2\text{O}_3$ (0001) (b) surfaces as a function of time: $[\text{Pb(II)}]_{\text{initial}} = 10^{-5}$ M, pH = 6.0, 0.01 M NaNO_3 . Observed x-ray reflectivity profiles (open grey circles) and LP-XSW Pb $L\alpha$ fluorescence yield profiles (open red diamonds), with calculated fits overlain (grey and red solid lines, respectively).

Fig. 8. Pb(II) (a) and Zn(II) (b) LP-XSW-FY profiles on *S. oneidensis* MR-1 biofilm-coated α - Al_2O_3 (1-102) surfaces as a function of time: $[\text{Pb(II)}]_{\text{initial}} = 10^{-5} \text{ M}$, pH = 6.0, 0.01 M NaNO_3 . Observed x-ray reflectivity profiles (open grey circles) and with calculated fits overlain (grey solid lines), LP-XSW Pb $L\alpha$ and Zn $K\alpha$ fluorescence yield profiles (open red and blue diamonds, respectively), with calculated fits overlain (red and blue solid lines, respectively). FY data for the short exposures (20-30 min and 3 hr) taken at the APS could be fit reasonably well, whereas the fits of FY data for the longer exposure samples (24 hr) taken at both the APS and SSRL are poorer and may indicate formation of a precipitate.

Fig. 9. **(a)** Pb L₃-edge normalized, background-subtracted k^3 -weighted EXAFS spectra and Fourier transforms (not phase-shift corrected) for Pb(II) sorbed on *S. oneidensis* MR-1 biofilm-coated α -Al₂O₃ (1-102) surfaces at an incident x-ray angle (0.1°) that probes Pb in the biofilms, and **(b)** Zn K-edge normalized, background-subtracted, k^3 -weighted EXAFS spectra and Fourier Transforms (not phase-shift corrected) of Zn(II) sorbed on *S. oneidensis* MR-1 biofilm-coated α -Al₂O₃ (1-102) surfaces at an incident x-ray angle (0.1°) that probes Zn in the biofilms. Solid lines = experimental spectra; dashed lines = fits.

Fig. 10. Site densities and pK_a values of carboxyl, phosphoryl, and hydroxyl/amine functional groups on bacterial cells from the literature. * Site concentrations are converted to g_{wet} using a conversion factor of 8:1 for wet to dry mass ratio (Borrok et al., 2005).

Fig. 11. The pH effect on Pb(II) partitioning on *S. oneidensis* MR-1 biofilm coatings on α -Al₂O₃ (1-102) surfaces.

Fig. 12. Schematic representation of ion partitioning at *S. oneidensis* MR-1 biofilm coatings on $\alpha\text{-Fe}_2\text{O}_3$ (0001) surfaces.

Table 1: Pb(II) distributions at *S. oneidensis* MR-1 biofilm-coated metal-oxide surfaces, pH = 6.0, exposure time = 3 hrs

Metal-oxide surface	Initial Pb(II) concentration (M)	LP-XSW-FY measured			Langmuir model prediction	
		% in the biofilm ^a	% at the surface ^b	Standard deviation	% at the surface ^c	% at the surface ^d
α -Fe ₂ O ₃ (0001)	10 ⁻⁴ M	>99	<1	<1	<1	<1
	10 ⁻⁵ M	>99	<1	<1	2	3
	10 ⁻⁶ M	74	26	1.5	11	20
	10 ⁻⁷ M	41	59	7.2	30	48
α -Al ₂ O ₃ (1-102)	10 ⁻⁴ M	>99	<1	<1	<1	2
	10 ⁻⁵ M	>99	<1	<1	2	7
	10 ⁻⁶ M	77	23	6.3	8	27
	10 ⁻⁷ M	48	52	5.9	14	40
α -Al ₂ O ₃ (0001)	10 ⁻⁵ M	>99	<1	<1	3	<1
	10 ⁻⁶ M	>99	<1	<1	6	<1
	10 ⁻⁷ M	>99	<1	<1	7	<1

^a Average percentage distribution in the biofilms measured from different positions of the sample or independent samples.

^b Average percentage distribution at the metal-oxide surface measured from different positions of the sample or independent samples.

^c Langmuir model prediction using the same site densities on the metal-oxide surfaces in Templeton et al. (2001)

^d Langmuir model prediction using site densities on the metal-oxide surfaces listed in Table 6.

Table 2: Zn(II) distributions at *S. oneidensis* MR-1 biofilm-coated metal-oxide surfaces, pH = 6.0, exposure time = 3 hrs

Metal-oxide surface	Initial Zn(II) concentration (M)	LP-XSW-FY measured		
		% at the biofilm ^a	% at the surface ^b	Standard deviation
α -Fe ₂ O ₃ (0001)	10 ⁻⁴ M	>99	<1	<1
	10 ⁻⁵ M	>99	<1	<1
	10 ⁻⁶ M	59	41	7.4
	10 ⁻⁷ M	55	45	1.9
α -Al ₂ O ₃ (1-102)	10 ⁻⁴ M	>99	<1	<1
	10 ⁻⁵ M	>99	<1	<1
	10 ⁻⁶ M	>99	<1	<1
	10 ⁻⁷ M	85	15	3.8
α -Al ₂ O ₃ (0001)	10 ⁻⁴ M	>99	<1	<1
	10 ⁻⁶ M	>99	<1	<1
	10 ⁻⁷ M	>99	<1	<1

^a Average percentage distribution in the biofilms measured from different positions of the sample or independent samples.

^b Average percentage distribution at the metal-oxide surface measured from different positions of the sample or independent samples.

Table 3: Pb(II) and Zn(II) distributions at *S. oneidensis* MR-1 biofilm-coated metal-oxide surfaces as a function of time, pH = 6.0, 0.01 M NaNO₃.

Metal-oxide surface	Initial [Pb(II)]	Initial [Zn(II)]	Exposure Time	% at the biofilm ^a	% at the surface ^b	Standard deviation	% at the surface by Langmuir Model predication
α -Al ₂ O ₃ (1-102)	10 ⁻⁵ M		20 min	>99 ^c	<1 ^c	NA	
α -Al ₂ O ₃ (1-102)	10 ⁻⁵ M		3h	>99	<1	<1	
α -Al ₂ O ₃ (1-102)	10 ⁻⁵ M		24h	54 ^c	46 ^c	NA	10.4 ^d
α -Al ₂ O ₃ (1-102)	10 ⁻⁶ M		20 min	>99	<1	<1	
α -Al ₂ O ₃ (1-102)	10 ⁻⁶ M		3h	77	23	6.3	
α -Al ₂ O ₃ (1-102)	10 ⁻⁶ M		20h	39 ^c	61 ^c	19.6	35.0 ^d
α -Al ₂ O ₃ (1-102)		10 ⁻⁵ M	30 min	>99	<1	<1	
α -Al ₂ O ₃ (1-102)		10 ⁻⁵ M	3h	>99	<1	<1	
α -Al ₂ O ₃ (1-102)		10 ⁻⁵ M	24h	75 ^c	25 ^c	NA	
α -Al ₂ O ₃ (0001)	10 ⁻⁵ M		30 min	>99	<1	<1	
α -Al ₂ O ₃ (0001)	10 ⁻⁵ M		3h	>99	<1	<1	
α -Al ₂ O ₃ (0001)	10 ⁻⁵ M		24h	82 ^c	18 ^c	NA	<1 ^d
α -Fe ₂ O ₃ (0001)	10 ⁻⁵ M		30 min	>99	<1	<1	
α -Fe ₂ O ₃ (0001)	10 ⁻⁵ M		3h	>99	<1	<1	
α -Fe ₂ O ₃ (0001)	10 ⁻⁵ M		20h	62 ^c	38 ^c	NA	5.0 ^d

^a Average percentage distribution in the biofilms measured from different positions of the sample or independent samples.

^b Average percentage distribution at the metal-oxide surface measured from different positions of the sample or independent samples.

^c LP-XSW-FY fits are poor and data can only be used for qualitatively interpretation, no standard deviation is available for these samples. The x-ray reflectivity data and fit of some samples show large discrepancy at angles larger than the critical angle of the metal-oxide substrates because the data were collected at SSRL beamline 11-2, where x-ray reflectivity configuration was not optimal at the time of data collection due to the limitations of the experimental set up.

^d Model prediction using modified Langmuir model as described in Section 4.3 using the site densities of metal-oxide surfaces presented in Table 6. In term of biofilms, 30% loss of biofilm functional groups was assumed and used for calculation metal partitioning for the long time exposure (20-24 hr) samples.

Table 4: Pb and Zn GI-EXAFS analysis for *S. oneidensis* MR-1 biofilm-coated α -Al₂O₃ (1-102) surfaces at an x-ray incidence angle (0.1°) that probes the metal ions probing in the biofilms

Metal ions	pH	Time	Atom	CN	R (Å)	σ^2 (Å ²)	ΔE_o (eV)	χ_{red}^2
Pb(II)	6.0	3hr	O	2.0(1)	2.38(1)	0.008	5.0	5.57
			O	2.2(1)	2.38(1)	0.010	4.8	2.75
			C	1.0(3)	3.37(3)	0.007		
Zn(II)	6.0	3hr	O	3.6(4)	1.98(1)	0.007	1.0	14.51
			O	3.4(2)	1.99(1)	0.006	1.4	8.75
			C	2.2(6)	2.93(2)	0.003		

Table 5:

Metal binding affinities and site densities of various functional groups for different type of bacteria strains

Bacteria type	Types of binding sites	Site concentrations (10 ⁻⁵ Mole/g _{wet})	Binding affinity ^a (Log K_{app_Pb})	Binding affinity ^b (Log K_{app_Zn})
<i>S. oneidensis</i> MR-1 (Ha et al., 2010)	Carboxyl	14.0	4.6	3.6
	Phosphoryl	4.1	4.1	3.7
<i>S. putrefacienns</i> (Claessens and Van Cappellen, 2007)	Carboxyl	–	–	4.1 (±0.2)
	Phosphoryl	–	–	5.5 (±0.1)
<i>B. subtilis</i> (G+) (Fein et al., 1997)	Carboxyl	12 (±2.0)	4.2	–
	Phosphoryl	4.4 (±0.3)	5.6	–
<i>B. licheniformis</i> (Daughney et al., 1998)	Carboxyl	8.88 (±3.8)	4.7 (±0.3)	–
	Phosphoryl	8.34 (±4.6)	5.7 (±0.7)	–
<i>C. metallidurans</i> (Guine et al., 2006)	Carboxyl	5.25 (±1.625) ^c	–	2.0 (±0.8)
	Phosphoryl	1.5 (±0.15) ^c	–	4.0 (±0.2)
	Hydroxyl/Amino	5.6 (±1.75) ^c	–	5.2 (±0.8)
<i>Enterobacteriaceae</i> (Ngwenya et al., 2003)	Carboxyl	6.25 (±0.88) ^c	3.9 (±0.8)	3.3 (±0.1)
	Phosphoryl	2.75 (±0.08) ^c	5.0 (±0.9)	5.1 (±0.1)
<i>Rhodococcus erythropolis</i> A 177 (Plette et al., 1996)	Carboxyl	6.3 ^c	–	1.9
	Phosphoryl	2.4 ^c	–	4.1
<i>A. macleodii subsp fijiensis</i> (Loaec et al., 1997)	EPS	–	5.15	4.39
Average values for natural and contaminated bacteria consortia (Ginn and Fein, 2008)	Phosphoester	10.0 (±1.7)	3.3 (±0.2)	–
	Carboxyl	9.0 (±3.0)	4.5 (±0.3)	–
	Phosphoryl	4.6 (±1.8)	6.5 (±0.1)	–
	Hydroxyl/Amino	6.1 (±2.3)	7.9 (±0.5)	–

^a Log K_{app_Pb} is the apparent binding affinity to Pb(II).

^b Log K_{app_Zn} is the apparent binding affinity to Zn(II).

^c Site concentrations are converted to g_{wet} using a conversion factor of 8:1 for wet to dry mass ratio (Borrok et al., 2005).

Table 6: Site densities and Pb(II) binding affinities of various functional groups in the biofilms and at three single crystal metal-oxide surfaces

Biofilms/metal-oxide surfaces	Types of binding sites	Site densities ^a (Log n)	Binding affinities ^b (Log K_{app_Pb})	pH _{pzc} (Point of zero charge)
<i>S. oneidensis</i> MR-1 biofilms	Carboxyl	-3.88	4.1 ^c	4.0 ^c
	Phosphoryl	-5.53	4.1 ^c	
α -Al ₂ O ₃ (0001)	M _{strong} ^d	-7.25 ^f	5.35 ^g	4.5 ⁱ
	M _{weak} ^e	-5.97 ^f	2.7 ^g	
<i>S. oneidensis</i> MR-1 biofilms	Carboxyl	-3.58	4.1 ^c	4.0 ^c
	Phosphoryl	-5.23	4.1 ^c	
α -Al ₂ O ₃ (1-102)	M _{strong} ^d	-5.60 ^h	6.0 ^g	5.1 ⁱ
	M _{weak} ^e	-5.29 ^h	3.55 ^g	
<i>S. oneidensis</i> MR-1 biofilms	Carboxyl	-3.08	4.1 ^c	4.0 ^c
	Phosphoryl	-4.73	4.1 ^c	
α -Fe ₂ O ₃ (0001)	M _{strong} ^d	-5.51 ^h	6.65 ^g	6.5 ⁱ
	M _{weak} ^e	-5.20 ^h	3.5 ^g	

^a Site densities (n, mol/m²)

^b Log K_{app_Pb} is the apparent Pb(II) binding constant.

^c Recalculated log K_{app_Pb} using a Langmuir model, with data from Ha et al. (2010).

^d M_{strong} represents the strongest binding site (MO) on the metal-oxide surface.

^e M_{weak} represents the weaker binding sites (M₂O and M₃O) on the metal-oxide surface.

^f Based on synchrotron-based XPS studies of the initial reaction of water with clean surfaces of α -Al₂O₃ (0001) (Liu et al., 1998), the percentage of singly coordinated, highly reactive defect sites is ~ 5%, whereas ~ 95% of the surface sites comprise less reactive doubly (M₂O) coordinated sites.

^g From Templeton et al. (2001)

^h Based on CTR studies by Trainor et al. (2002) and Trainor et al. (2004), the percentage of strongest binding site MO on reactive α -Fe₂O₃ (0001) and α -Al₂O₃ (1-102) surfaces is 33%, whereas the percentage of the weaker binding sites (M₂O and M₃O) is 67%. The total site densities are from Templeton et al. (2001).

ⁱ From Wang et al. (to be submitted)