XAFS and XSW study of the distribution of Pb(II) sorbed to biofilms on α-Al₂O₃ and α-FeOOH surfaces

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Biofilms form ubiquitous organic coatings on mineral surfaces in soil and aquatic environments. In order to understand the chemical speciation of heavy metals (such as Pb) sorbed to minerals colonized by bacteria, it is essential to evaluate the coupled biotic and abiotic sorption by such heterogeneous systems. We have used EXAFS spectroscopy to quantitatively determine the partitioning of Pb between biofilms (*Burkholderia cepacia*) and the reactive sites on powdered α-Al₂O₃ and α-FeOOH. We have also used x-ray standing wave (XSW) methods to study the distribution of Pb sorbed to biofilms grown on the surface of single crystal α-Al₂O₃ (1-102). The XSW data show that Pb occurs dominantly at the α-Al₂O₃ (1-102) surface at low [Pb]$_T$ and is more distributed within the biofilm at higher [Pb]$_T$. The Pb L$_{III}$ EXAFS data show that Pb is partitioned more strongly to the α-FeOOH surface than the α-Al₂O₃ surface relative to the biofilm. However, the fraction of Pb sorbed to the α-Al₂O₃ mineral surface relative to the biofilm may increase as Pb coverage increases.

Keywords: Biofilm, Pb(II), XSW, alumina, goethite

1. Introduction

Pb is a common environmental pollutant whose transport and biogeochemical cycling in soils and aquatic environments can be dramatically affected by sorption and precipitation reactions on mineral surfaces. Recent efforts to characterize Pb speciation in contaminated soils and mine wastes have focused on identifying the speciation of Pb incorporated into solid phases (i.e., galena, anglesite, pyromorphite, (hydro)cerrusite, plumbojarosite etc.), Pb bound to organic compounds such as humic and fulvic acids, and Pb sorbed to mineral surfaces (i.e., birnessite, goethite, aluminum hydroxides) (e.g. Manceau et al., 1992; O'Day et al., 1998; Bargar et al., 1997a,b; Ostergren et al., 1998, 1999; Morin et al., 1998). However, fine-grained mineral particles in soils and aquatic environments are often intimately associated with organic matrices such as biofilms. Thus, it is essential to study the coupled biological and inorganic sorption of Pb by biofilm-coated minerals to develop more robust geochemical

models capable of predicting Pb transport and bioavailability in a variety of geochemical environments.

Biofilms form metabolically active organic surface coatings in which bacterial microcolonies are surrounded by highly hydrated extracellular polymers (e.g., polysaccharides). Biofilms may alter the surface reactivity of soil minerals (e.g., by blocking surface sites), may compete with the mineral surface for Pb uptake, or may create "microenvironments" in which the accumulation of both organic and inorganic ligands may enhance the precipitation of Pb phases not predicted thermodynamically for the bulk solution conditions. The relative contributions of biotic vs. abiotic factors to trace metal sorption will depend on the local conditions (Eh, pH, alkalinity, ionic strength), the availability of reactive surface sites on the mineral surface and the distribution of organic functional groups (i.e., carboxyl, phosphoryl, hydroxyl) within the biofilm. Since the adsorption of metal ions to microbial cells may be similar in nature to metal adsorption on oxide mineral surfaces (i.e., controlled by the speciation of the metal and the type of available surface sites; Warren and Ferris, 1998; Daughney and Fein, 1998, Collins and Stotszky, 1992), these processes may be investigated using similar spectroscopic techniques (i.e., EXAFS spectroscopy) to those already employed in mineral surface studies. For example, EXAFS spectroscopy has recently been used to study the binding of Pb to carboxyl and phosphoryl functional groups on fungal cell walls (Sarret et al., 1998).

In an initial study, we have used EXAFS and XSW techniques to identify Pb sorption complexes and precipitates on biofilm-coated mineral surfaces. In particular, we have combined these techniques to quantify the partitioning of Pb between the biofilm and the powdered or single-crystal mineral surfaces. Our biofilms were produced by culturing *Burkholderia cepacia*, a chemoorganotroph that forms biofilms dominated by exopolymer under nutrient limited conditions (Nelson et. al, 1995). The chosen mineral substrates have well-defined surface properties and serve as models for environmentally important phases; α-Al₂O₃ is used as analog for complex aluminum oxides, hydroxides, aluminosilicates and clay minerals found in natural sediments and α-FeOOH is a ubiquitous reactive mineral phase found in oxidized soils. Differences in the chemical reactivity of the α-Al₂O₃ and α-FeOOH surfaces allow us to compare the competitive sorption between the biofilm and the two oxide minerals.

2. Materials and Methods

2.1 Experimental design and data collection

Biofilms of *Burkholderia cepacia* were grown on mineral powders of alumina (α-Al₂O₃), laboratory synthesized goethite (α-FeOOH), and single crystal α-Al₂O₃ (1-102), using minimal media with a sufficiently low concentration of inorganic nutrients (i.e. sulfate, carbonate, phosphate) to avoid the homogeneous precipitation of Pb phases (Murgel et. al., 1991). Sorption samples of the biofilm-mineral aggregates were prepared by base titration to pH 6 for 50 ml of a 2.5 or 5 g/L biofilm-mineral suspension, [Pb]$_T$ = 22.5 μM, and 0.01M NaNO₃ as a background electrolyte. CO₂ was excluded by continuously sparging with Ar gas. The biofilms grown on single-crystal substrates were immersed in a 1 or 30 μM Pb(NO₃)₂ solution (pH 6) for 16 hours, removed from solution under a jet of N₂ and transferred to a teflon holder (constant 95% humidity) positioned on a grazing-incidence rail to collect X-ray standing wave spectra. All XAFS and

XSW data were collected at the Stanford Synchrotron Radiation Laboratory on wiggler beamlines (3 GeV, 30-100 mA, detuned 30-60% at 13600 eV) using an unfocused beam. The XSW data for *B. cepacia* biofilms grown on single-crystal α-Al_2O_3 (1-102) were collected on beamline 4-2 using Si(220) monochromator crystals and a 13-element high-throughput Ge-detector. The XSW data were collected from 0 to 500 mdeg with a vertical beam size of 0.15 mm in order to match the "footprint" of the beam with the crystal length (3 cm). The data were normalized to the maximum fluorescence signal (Fig. 2). We collected Pb L_{III} EXAFS spectra on biofilm-coated mineral samples loaded in 3 mm teflon holders on beamline 4-3 using Si(220) or Si(111) monochromator crystals and a Stearn-Heald type "Lytle" detector filled with Xe gas. An As (6T) filter was used to attenuate elastic scattering, and the energy was calibrated using a Pb-metal foil; the first inflection of the Pb metal L_{III} absorption edge was set to 13055 eV and E_0 for the EXAFS was defined as 13070 eV (15 eV above the first inflection point).

2.2 Data analysis

We have applied an empirical linear-combination fitting procedure to the $k^3\chi(k)$ functions for our biofilm-coated mineral samples, using the unsmoothed, background-subtracted, spline-fit, k^3-weighted Pb-model EXAFS spectra, to quantitatively separate the contribution of the Pb uptake by the biofilm from Pb sorption to the mineral (α-Al_2O_3 or α-FeOOH) surface. The fraction of total Pb in each component is given directly by the fraction of the corresponding model spectrum contributing to the fit. This technique is relatively robust when using unique EXAFS "fingerprints" for the major components of the composite spectrum and in our system, because the EXAFS spectra of Pb(II) sorption complexes on Fe and Al hydroxides are quite different from those of Pb(II) precipitates (i.e. Pb-phosphate, carbonate and sulfate; not shown) or Pb sorbed to the organic biofilm exopolymer matrix (Fig. 1a). Application of the linear least-squares fitting procedure to known mixtures of Pb model compounds provides results accurate to within ±25% of the stated atomic fractions present for the components and is capable of identifying contributions greater than 10% (Ostergren et al., 1998).

3. Results and Discussion

We obtained good quality EXAFS spectra of Pb (1 to 10 µM) sorbed to bacterial cells and exopolysaccharide, to use as "endmember spectra" in conjunction with previously published EXAFS spectra of Pb sorbed to alumina and goethite at low surface coverage (i.e. ~1 µmol/m^2) (Bargar et al, 1997a,b). These endmembers -- Pb sorbed to the biofilm and Pb sorbed to α-Al_2O_3 or α-FeOOH -- can be quantitatively compared to our EXAFS spectra of Pb (1 to 30 µM) sorbed to biofilms grown on our mineral substrates. Using a two-component linear-combination fitting procedure, we can achieve good fits for our EXAFS spectra of Pb sorbed to biofilm-mineral aggregates, which provide estimates of the partitioning of Pb between the mineral surface and the biofilm (Fig. 1).

In the Al_2O_3 system, Pb is dominantly sorbed to the exopolymer (84%) compared to the mineral surface (16%) at low surface coverages of Pb ($\leq$1.1 µmol/m^2). At higher [Pb]$_T$:solid ratios, the uptake of Pb and thus the total surface coverage on the biofilm-mineral aggregate increases ($\leq$2.2 µmol/m^2), and the fraction of Pb sorbed to the mineral surface

(26%) may have slightly increased relative to the fraction of Pb sorbed to the biofilm (74%) (Fig. 1 b,c). Given the small changes in metal paritioning indicated by our fitting procedure (Fig. 1 b,c) and the possible errors in the accuracy of the fits for our composite spectra, additional data points over a larger range in surface coverages or [Pb]$_T$:solid ratios are necessary to confirm this trend.
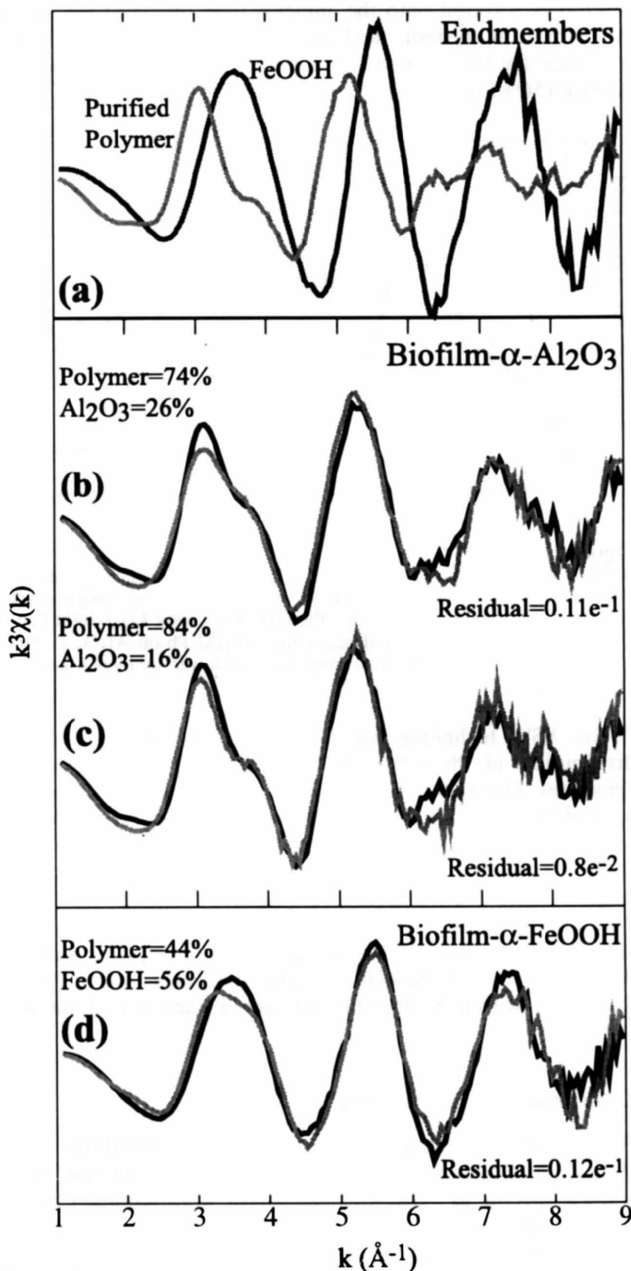

Figure 1
(a) Representative endmembers for two component fits: Pb sorbed to purified exopolymer (grey) and Pb sorbed to FeOOH (black). (b)-(d) Pb L_{III} EXAFS (black) and two-component linear-combination fitting results (grey) for biofilm-coated minerals, including fit residual (no units). (b) Pb sorbed to biofilm-coated α-Al_2O_3 powder at pH 6, $\Gamma \leq 2.2$ µmol/m^2, (c) Pb sorbed to biofilm-coated α-Al_2O_3 powder at pH 6, $\Gamma \leq 1.1$ µmol/m^2, (d) Pb sorbed to biofilm-coated α-FeOOH at pH 6, $\Gamma \leq 1$ µmol/m^2.

In the α-FeOOH system, we have collected EXAFS spectra for a biofilm-goethite aggregate with a low surface coverage of Pb ($\leq$1 µmol/m^2). Comparison of our spectra of Pb sorbed

to biofilm-coated goethite to that of Pb sorbed on clean goethite (Fig. 1a,d) shows a significant dampening of the oscillations beyond $k=8\text{Å}^{-1}$ and a loss of the second shell in the Fourier transform (Fourier transform and $k=8$ to 12Å^{-1} EXAFS not shown). This second shell feature in the Pb-goethite EXAFS spectra has previously been attributed to Fe next-nearest neighbors (Bargar et al. 1997b). Nevertheless, the spectra for the biofilm-coated goethite still more closely resembles sorption onto the mineral than observed for the α-Al_2O_3-biofilm system, and our fit of the data ($k=1$-9Å^{-1}) indicates that Pb is sorbed to a greater degree on the goethite surface (56%) than on the biofilm (44%) (Fig. 1d).
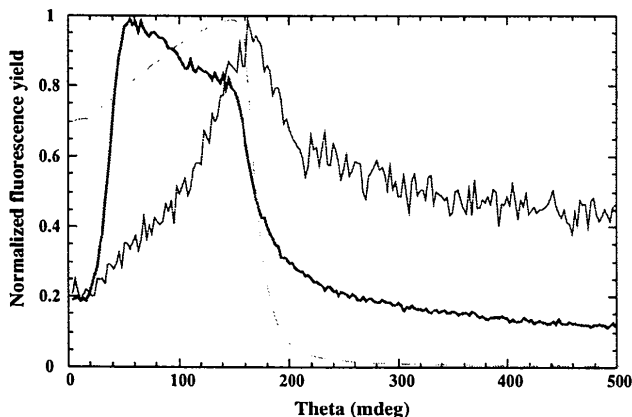

Figure 2
XSW results, plotted as normalized Pb L_{III} fluorescence yield as a function of incidence angle (theta) for biofilms on single-crystal substrates. Dashed line represents the reflectivity (log I_1/I_0), light line is 1 μM Pb on Al_2O_3 (1-102) and heavy line 30 μM Pb on Al_2O_3 (1-102). Note shift in maximum fluorescence yield to lower incidence angle at higher $[Pb]_T$.

The XSW technique can be used to probe the vertical distribution of Pb within biofilms grown on single-crystal substrates. Our x-ray standing wave (XSW) data for Pb sorbed to biofilms on α-Al_2O_3 (1-102) indicate that Pb is located predominantly at the mineral surface at low $[Pb]_T$, since a sharp maximum in the fluorescence yield is observed at the critical angle. In contrast, at higher $[Pb]_T$ the maximum fluorescence yield is measured at an incidence-angle significantly below the critical angle (Fig. 2). This shift in the maximum fluorescence yield indicates that Pb is distributed within the biofilm (i.e. above the mineral surface) at higher $[Pb]_T$.

4. Summary and Conclusions

We have obtained EXAFS spectra for low concentrations (1 to 10 μM) of Pb(II) sorbed to biofilms and we can use these EXAFS spectra as "fingerprints" to fit multi-component Pb spectra and thus to quantify the partitioning of Pb between the biofilm and the underlying mineral surface. Under our experimental conditions, linear-combination fitting of the Pb L_{III} EXAFS spectra shows that Pb(II) sorption to biofilm-coated oxide minerals is primarily dominated by sorption to the biofilm relative to α-Al_2O_3 but is more equally distributed between the biofilm and α-FeOOH. This is consistent with the greater reactivity and Pb uptake observed for goethite (α-FeOOH) relative to alumina (α-Al_2O_3) at pH 6-7 in biofilm-free systems.

Contrasting the single-crystal XSW data and powder EXAFS results for the α-Al_2O_3 system highlights the importance of quantifying the abundance and types of reactive sites available

on the biofilm vs. the mineral surface in predicting the partitioning of Pb. From our XSW results, it appears that Pb(II) sorbs preferentially to the α-Al_2O_3 (1-102) surface at low $[Pb]_T$, and that Pb is increasingly distributed within the biofilm at higher $[Pb]_T$. In contrast, for Pb(II) sorbed to the biofilm-coated alumina powders, our fitting of the EXAFS spectra shows that the majority of the Pb(II) is sorbed by the biofilm at the lower Pb surface coverage and that the fraction of Pb(II) sorbed to the mineral surface relative to the biofilm may increase slightly at higher loadings of Pb. Our preliminary interpretation of the EXAFS results is that the biofilm has a greater affinity for Pb than the majority of the available surfaces on the powdered α-Al_2O_3. The preferential affinity of the mineral surface observed in the XSW data may be explained by previous work by Bargar et. al. (1996), which shows that Pb binds more strongly to the (1-102) surface than other α-Al_2O_3 surfaces such as the (0001) surface. Thus we suggest that although Pb sorbs preferentially to the α-Al_2O_3 (1-102) surface at low Pb concentrations in the single-crystal experiments, Pb(II) sorption is dominated by the biofilm in the more heterogeneous powder system because the less reactive (0001) surface (or its hydroxylated equivalent) should be prevalent in the α-Al_2O_3 powders. To test this hypothesis, additional XSW experiments are needed to confirm whether or not Pb(II) preferentially sorbs to the biofilm relative to the α-Al_2O_3 (0001) surface at low $[Pb]_T$.

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