

## Pb Speciation at Sapphire and Hematite Surfaces Associated with Bacterial Biofilms

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Metal sorption to mineral surfaces is strongly influenced by the structure and reactivity of the substrate. However, we have little information on how mineral surface structure and chemistry may influence the development of bacterial biofilms, nor how those biofilms may subsequently affect the sorptive properties of the mineral substrate. Our goal is to examine the partitioning of Pb(II) between metal-oxide surfaces and associated bacterial biofilms, to characterize the speciation of Pb(II) within the biofilm and to determine how the development of a biofilm may affect the speciation of Pb that is sorbed to the underlying mineral surface.

We have developed a coupled X-ray standing wave (XSW) and Grazing-incidence x-ray absorption fine structure (GIXAFS) spectroscopy approach for studying heavy metal sorption to biofilms formed on single-crystal surfaces. An integrated XSW and GIXAFS approach is useful for (1) quantitatively measuring changes in the partitioning of metals between the crystal surface and the biofilm as a function of environmental variables such as metal concentration or pH and (2) characterizing changes in the speciation of metals sorbed to the crystal surface vs. the biofilm layer. We have grown biofilms of *Burkholderia cepacia* on polished single-crystals of  $\alpha$ -Al<sub>2</sub>O<sub>3</sub> (1-102) and (0001) and  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> (0001) and reacted these samples with Pb(II) at concentrations ranging from 0.1 micromolar to 300 micromolar at pH 6. XSW data were collected in order to quantitatively model the Pb fluorescence yield profiles and obtain partition ratios for Pb sorbed to the mineral surface relative to Pb sorbed to the biofilm. GIXAFS measurements were collected on a subset of samples to separately examine the speciation of Pb associated with the biofilm and the mineral surface.

Qualitative inspection of the XSW data show that the partitioning of Pb between the biofilm and the mineral surface is sensitive to Pb concentration and that Pb is increasingly partitioned into the biofilm at higher Pb concentrations on all surfaces studied. Quantitatively, we observe a strong dependence of the partitioning and speciation of Pb upon the structure and chemical composition of the mineral substrate. The threshold Pb concentration at which Pb is equally partitioned

between the biofilm and the mineral differs dramatically for the three crystal substrates. For example, when Pb is sorbed to isostructural  $\alpha$ -Al<sub>2</sub>O<sub>3</sub> (0001) vs.  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> (0001), sorption is dominated by the biofilm on  $\alpha$ -Al<sub>2</sub>O<sub>3</sub> (0001) surfaces at concentrations greater than 0.1 micromolar whereas sorption is dominated by the  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> (0001) surface relative to the biofilm at all Pb concentrations less than 150 micromolar. Overall, the degree of sorption to the mineral surface vs. the biofilm at a given Pb concentrations consistently follows the order  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> (0001) >  $\alpha$ -Al<sub>2</sub>O<sub>3</sub> (1-102) >  $\alpha$ -Al<sub>2</sub>O<sub>3</sub> (0001), demonstrating that the more reactive mineral surfaces compete more effectively with the biofilm during Pb sorption.

As changes in the partitioning of Pb between the biofilm and the three mineral surfaces occur, we have characterized the changing nature of the dominant sorption complexes. GIXAFS data for Pb(II) sorbed to  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> (0001) shows direct (inner-sphere) bonding of Pb to FeO<sub>6</sub> octahedra at all Pb concentrations, but the local structure of the sorption complex differs significantly at low and high sorption densities as unique crystallographic sites are successively populated. Similarly, the local structure of the Pb sorption complex on the  $\alpha$ -Al<sub>2</sub>O<sub>3</sub> (1-102) surface differs from results obtained in biofilm-free systems, indicating that the biofilm affects mineral surface sites that are involved in Pb binding. Within the biofilm itself, the dominant speciation of Pb(II) appears to be strongly affected by the chemical composition of the crystal substrate. For example, there is significant formation of a Pb-phosphate phase within the biofilms formed on  $\alpha$ -Al<sub>2</sub>O<sub>3</sub> surfaces, whereas Pb is primarily sorbed to carboxyl and phosphoryl functional groups within the biofilms formed on  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub>, even at significantly higher metal loadings.

Our studies demonstrate that the reactivity of the mineral surface is a controlling factor in the initial partitioning of Pb from aqueous solutions. Moreover, synergistic effects between the biofilm and the mineral surfaces lead to a scenario in which (a) the development of a biofilm changes the nature of the preferential binding sites on the mineral surface while (b) the chemical identity of the mineral substrate influences the speciation of the metal within the biofilm.