Competitive Adsorption of Cd to Bacterial Cell Wall and Mineral Surfaces – XAFS study

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Introduction

Surface and sub-surface metal contamination of environment is a universal problem of immense complexity and great global concern. Since these metal contaminants form chemical complexes, they can be present in many forms ranging from a rather inert state to a highly mobile, toxic and reactive phase. Their transport properties and toxicity are influenced by their oxidation states, solubility and sorption processes which in turn is strongly correlated to the chemical and physical attributes of the surrounding medium. Solid-solution interface reactions play a very important role in their transport and bioavailability. Adsorption to mineral surfaces has been relatively well studied due to their amenability to a number of structural characterization methods. But the effect of bacteria and the extra-cellular material associated with them has not been extensively studied, partly due to ignorance about their potential impact and partly due to the complexity involved in studying the mixed bacteria-mineral systems. Many recent investigators have suggested that microorganisms play an important role in metal mobility. In addition, the microenvironment at and adjacent to actively metabolizing cell surfaces can be significantly different from the bulk environment. Thus, to develop a precise mechanistic understanding of the exact reactions taking place, the spatial distribution and chemical speciation of contaminants in presence of both mineral and microbe must be characterized at sub-atomic resolution. Understanding and modeling metal transport in the environment is not only vital in determining the environmental fate of these contaminants and their relative risk but also in developing remediation strategies as the final goal.

To this end EXAFS is being used to determine the binding sites of adsorbed Cd to Fe oxide (HFO) minerals and isolated cell walls of *Bacillius subtilis.* The competitive adsorption between biomass and Fe oxides is under investigation as a function of pH, metal concentration, and metal:biomass:oxide ratio. Results of this investigation will be used for precise identification of adsorption reaction in surface complexation modeling as well as to give insight on the fundamental interactions between the metal ion, the available ligands, and on the prospects of modeling them in a unified thermodynamic model.

Methods and Materials

Bacterial growth and wash procedure were followed as described in Fein et al, (1997, 2001) [1]. A plated colony was transferred from the experimental plate to a sterile 7mL TSB + 0.5% yeast extract broth tube under the laminar flow hood which was then incubated for 24 hours at 32 C in an incubator/shaker. Broth tube was tranferred to 1L of TSB + 0.5% yeast extract under laminar flow hood and incubated for another 24 hours at 32 C in an incubator/shaker. Cells thus harvested during stationary growth phase were washed first in 0.1 M NaClO4 electrolyte followed by a rinse with 0.03 M HNO3 and five rinses in fresh 0.1 M NaClO4 electrolyte. Washed bacteria were suspended in 0.1 M NaClO4 electrolyte to form parent solution of 10g/L of bacteria (wet weight). 1000 PPM of parent Cd solution (99.99% pure) and chemically pure HFO (both obtained from Alfa-Aldrich) was used. XRD was perfomed on HFO to check its phase purity. Samples were prepared by adding appropriate amount of dissolved metal from the aqueous 1000 PPM Cd standard to attain the required final Cd concentration. 2g/L HFO was used in all the samples. This number was obtained using ICP-AES study to match 10g/L of biomass concentration such that Cd would be equi-distributed on biomass and HFO sites in the mixed samples. The pH was adjusted using 1.0 M NaOH or HNO3. After that samples were allowed to equilibrate for 2 hours. The pH was adjusted after every half an hour and the final pH was recorded. A detailed sample matrix is shown in Table 1.

XAFS measurements were carried out at Materials Research Collaborative Access Team (MRCAT) sector 10-ID beamline at APS. This is an undulator beamline with scanning monochromator. 111 plane of cryogenically cooled Si double crystal monochromator was used to tune Cd K edge (26.71 KeV). A Pt coated harmonic rejection mirror was used to eliminate higher harmonics. Defining slits were used to get a spot size of 700*700 microns. To avoid radiation damage, sample was moved after every scan to let the beam hit at slightly different positions. Further, near-edge structure before and after irradiation was compared, which is a quite sensitive probe for radiation damage. 13 element energy dispersive multi-element detector (Canberra electronics) was used for measuring all these samples. Measuring mineral samples would have been almost impossible for 0.3 PPM Cd-HFO and Cd-bio-HFO samples otherwise. Two folds of household Al foil was used to filter out Fe fluorescence peak in the mineral and mixed samples. Measurements were done in tapered mode to obtain less than 15% change in Io. Cd foil was measured in parallel for absolute energy calibration and edge alignment of different scans.

Results

A comprehensive study is being done at pH 5.9 and then one of the four concentrations will be picked up to scan along the pH range. A table for sample matrix is shown below [see Table 1].

This pH was chosen to match previous study. It also avoids the experimental hassle (sample preparation part) of working above pH 6.5 and still be in mid pH range which has its own environmental importance. The Cd concentration covers two orders of magnitude.

pH = 5.9

Cd-BIO(10g/l)	*Cd-HFO(2g/l)*	*Cd-HFO-BIO (2:10 g/l)*
30 ppm	30 ppm	30 ppm
10 ppm	10 ppm	10 ppm
3.0 ppm	3.0 ppm	3.0 ppm
0.3 ppm	0.3 ppm	0.3 ppm

Table 1: Sample matrix for the series of experiment.

Measurement of first column shows a difference in binding mechanism as a function of concentration.[see Fig 1].

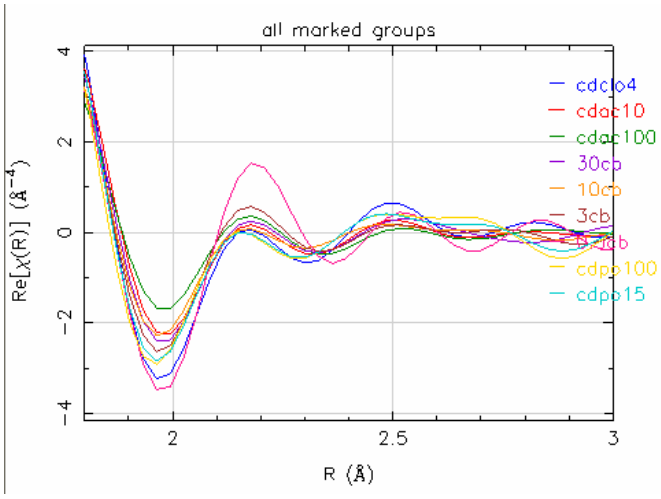

Fig 1: Zoomed version of Real Part of the K^3 FT for 30, 10, 3.0 and 0.3 ppm samples overplotted with solution standards CdClO4, CdAc (1:10 and 1:100) and CdPO4 (1:15 and 1:100).

It can be easily seen here that 30, 10 and 3.0 ppm samples lies within the boundary drawn by the standards having smallest (CdCl04) and highest amount of carbon. But the behavior shown by 0.3 ppm Cd-bio sample is remarkably different. This qualitative result is in agreement with Fein et al.(1997) where they report a one-site model and two-site model for high and low metal:bacteria ratio respectively. This qualitative analysis, however, has to be confirmed by a more rigorous fitting model to determine the exact contribution of carboxylic and phosphate sites.

Measurements of first row confirm that the mixed system is not a linear combination of the Cd-Bio and Cd-HFO samples.[see Fig 2]. It implies that presence of either bacteria or (and) mineral interferes with Cd uptake of the other.

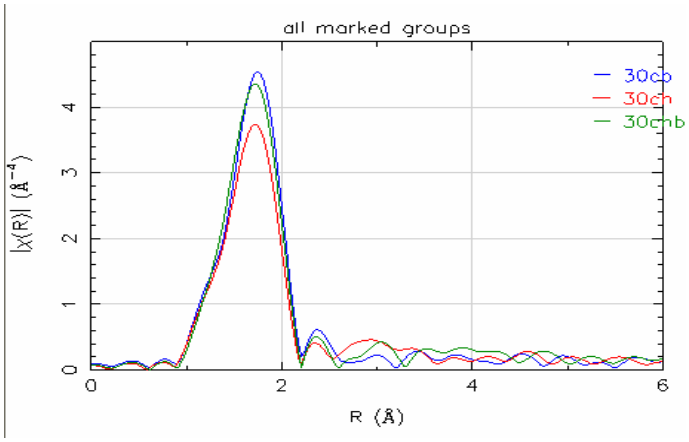

Fig 2 K^3 FT Mag of 30 PPM Cd adsorbed to biomass, HFO and mixed Bio-HFO systems.

A systematic change in C peak at about 2.2 A can be observed. However the Fe peak at about 3.0 A is smeared out for the Cd-HFO sample. This suggests a strong possibility of a highly disordered multiple sites in HFO. Lower O shell amplitude in Cd-HFO sample also suggests the same. Multiple sites in HFO has also been previously reported in literature.[4]

Discussion

Implication of this study is threefold: a) study of the Cd-biomass samples as a function of Cd concentration will test if the binding mechanism of biomass changes with change in metal concentration at a constant pH. If this mechanism is fairly constant over a range of two orders of magnitude, the reaction constants predicted by thermodynamic models would be pretty robust over the entire range. It would simplify the implementation of remediation strategies as one would not need to worry about metal concentration in the contaminated fields which often varies from site to site. However, if changes in metal concentration fundamentally changes the way they bind to the bacterial ligands, it must be known and stratified concentration ranges and their corresponding reaction constants must be known for developing effective remediation strategies. This will address the key scientific question, what surface complexation modeling approaches are best suited for accounting for metal adsorption in natural consortia of bacteria? This is based on the assumption that all bacterial surface exhibit similar binding mechanism, which needs to tested and verified. b) Studying the mixed mineral-microbe system will answer whether or not the binding mechanism of bacteria to metals in mineral-microbe systems similar to its binding mechanism for single species? c) and finally how the competitive adsorption of metals to bacterial cell walls versus mineral surfaces vary with change in pH conditions.

In order to answer these rather complex questions, a thorough understanding of the local coordination environment of Cd in aqueous environment is required. To achieve this, a series of

solution and solid standards have been measured and their fitting is in progress. Bacterial cell walls are now known to have carboxyl and phosphoryl binding ligands at this pH. [3] No appropriate standard was found for Cd-HFO samples. This problem becomes particularly complex due to the amorphous nature of HFO. Nevertheless, HFO was chosen because it is closest to the natural environmental conditions.

Enough scans were taken on each sample to collect 2-3 million effective counts. The edge energies were chosen at the inflection point. ATHENA was used to normalize the data and remove the background with k-weight 2. Transform range of 2.1 A -1 to 9.4 A-1 was used in ATHENA to Fourier Transform the data. FT k-weight of 3, R_bkg value of 1.2 and Hanning window function with dk 1.0 was used for all these transforms. Standards have been fit with FEFFIT using FEFF 8.2 for theory. [6, 7, 8] Rigorous (quantitive) EXAFS modeling and fitting for the unknown samples is in progress. Comparison with solution standards and preliminary analysis suggests that binding mechanism of Cd-bio is fairly constant over the range 30 PPM to 3 PPM but a dramatic change is seen in the 0.3 PPM sample.[see Fig 1] It is possible that an altogether different site dominates the adsorption mechanism at sub ppm level. This result is in agreement with previous macroscopic study [1] and needs to be tested at molecular level. However nothing can be said with certainty without full quantitive analysis. Similar study of Cd-HFO also suggests a strong possibility of multi site system. [Fig not shown]. Comparison of 30 PPM Cd-bio and Cd-HFO samples with 30 PPM mixed mineral-microbe sample shows that the mixed sample is not just a linear combination of the two components. It clearly indicates that the presence of bacterial and mineral sites affect metal uptake of each other. Unfortunately we are neglecting the effect of possible biomineralization for this study which could be another explanation for the same.

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