The pH-dependence of U interaction with bacterial cell walls measured by X-ray Absorption Spectroscopy

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Abstract

Metal mobility in subsurface water systems involves the complex interaction of the metal, the fluid, and the mineral surfaces over which the fluid flows. This mobility is further influenced by metal adsorption onto bacteria and other biomas in the subsurface. To better understand the mechanism of this adsorption as well as its dependence on the chemical composition of the fluid, we have performed a series of metal adsorption experiments of aqueous uranyl (UO₂⁺) to the gram-positive bacterium B. subtilis in the presence and absence of carbonate along with X-ray Absorption Spectroscopy (XAS) to determine the binding structures at the cell surface. In this paper we demonstrate an approach to the XAS data analysis which allows us to measure the partitioning of the adsorption of uranium to hydroxyl, carboxyl (or carbonato), and phosphoryl active sites at the cell surface.

Constraints and restraints in IFEFFIT

IFEFFIT has two ways of incorporating prior knowledge into a fitting model:

Constraint: An assertion about the value of a fitting parameter or a fixed relation between two or more parameters used in the fit.

Restrain: An expression placing a soft limit on the range of values available to one or more fitting parameters. The restraint is added in quadrature to the fitting metric \( \chi^2 \).

The parametric terms \( \{X, S, B, \sigma_0, E_1, E_2, C_1, \ldots \} \) need not, themselves, be the variables in the fit. Rather, they are expressed in terms of the variables in the fit.

Constraints and restraints used in the B. subtilis fits

1. Number of axial oxygen atoms fixed to 2. Uranyl complexes are always bound to 2 axial oxygens at about 1.96 Å.
2. Number or equatorial oxygen fixed to 6. This number was floated freely, fixed to 5, fixed to 6, restrained to 5, and restrained to 6. The data consistently preferred 6 equatorial oxygens, thus the value of 6 was asserted in the final fits.
3. Short equatorial oxygen atoms (R=2.27 Å) are associated with phosphoryl complexation.
4. Long equatorial oxygen atoms (R=2.42 Å) are associated with hydroxyl or carboxyl (or carbonato) complexation.
5. \( N_x - 6 \leq N_{eq} \leq N_x \)
6. The number of phosphorus scatterers is constrained to be the same as the number of short equatorial oxygens.
7. The number of carbon scatterers is restrained to be no more than half the number of long equatorial oxygens.
8. \( E_1 \) is constrained to be the same for all paths. This constraint can be lifted by changing \( E_1 \) to guess for each, thus giving the uranium an independent \( E \).
9. The carboxyl/carbonato complex is treated as a rigid unit, direct can be guessed to distinguish between carboxyl and carbonato ligations.

Fits were performed using theoretical standards from FEFF and the Artes program which uses IFEFFIT as its back-end). The fitting model allows the equatorial oxygens to partition between phosphoryl, carboxyl/carbonato, and hydroxyl/hydroxylation ligands. The best fit was obtained by also allowing sodium scatterers at about 4 Å.

The main distinction between the pH~4.6 samples and the higher pH samples was the number of phosphoryl complexation sites.

The pH-dependence of U interaction with B. subtilis culture media was measured by ICP-OES. These absorption data were modeled as electrostatic interactions between the charged bacterial surface and the aqueous U ion using FITEQL. This model identifies multiple binding sites tentatively associated with phosphoryl and carboxyl active sites on the cell wall and, for samples exposed to atmosphere, a surface carbonato complex.

Fitting results

<table>
<thead>
<tr>
<th>pH, CO₂</th>
<th>Phosphoryl</th>
<th>Carboxyl(carbonato)</th>
<th>Hydroxyl</th>
<th>Bond</th>
<th>length (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH=4.60, open</td>
<td>0.18 (78)</td>
<td>1.18 (79)</td>
<td>2.03 (70)</td>
<td>axial oxygen</td>
<td>1.771 (4)</td>
</tr>
<tr>
<td>pH=4.60, open, w/Ca</td>
<td>1.90 (64)</td>
<td>2.45 (86)</td>
<td>2.464 (8)</td>
<td>short equatorial</td>
<td>2.370 (9)</td>
</tr>
<tr>
<td>pH=4.60, no CO₂</td>
<td>0.96 (86)</td>
<td>1.54 (88)</td>
<td>3.578 (6)</td>
<td>long equatorial</td>
<td>2.464 (8)</td>
</tr>
<tr>
<td>pH=4.60, no CO₂</td>
<td>0.96 (86)</td>
<td>1.54 (88)</td>
<td>3.578 (6)</td>
<td>phosphorus</td>
<td>2.903 (36)</td>
</tr>
<tr>
<td>pH=4.60, no CO₂</td>
<td>0.96 (86)</td>
<td>1.54 (88)</td>
<td>3.578 (6)</td>
<td>carbon</td>
<td>4.014 (28)</td>
</tr>
</tbody>
</table>

Consistent with earlier results, we find that phosphoryl complexation decreases as pH increases. The thermodynamic modeling indicates the presence of an inorganic uranyl carbonate or calcium uranyl carbonate complexation.

We do not see any indication of Ca in the Ca-bearing sample. Indeed, the fitting model for all samples used a Ca atom at about 4 Å for fits of the quality shown above.

Distinguishing a carboxyl ligand from an inorganic carbonato complex is tricky. They differ by the replacement of the C atom at about 4.35 Å with an O at about 4.2 Å. The spectral weight at that distance is slight and both carbon and oxygen are weak scatterers.