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

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Abstract

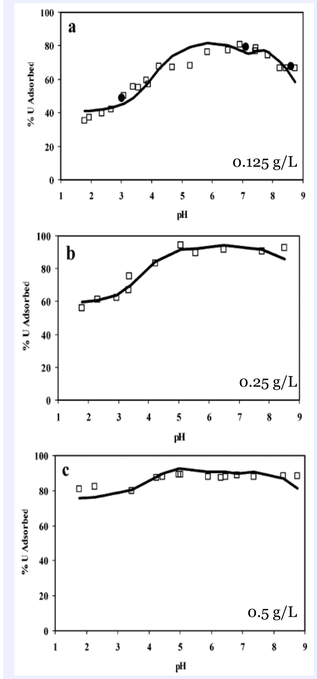
Metal mobility in subsurface water systems involves the complex interaction of the metal, the fluid, and the mineral surfaces over which the fluid flows. This mobility is further influenced by metal adsorption onto bacteria and other biomass in the subsurface. To better understand the mechanism of this 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 $(UO_2)^{2+}$ 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.

The failure of storage facilities containing the contaminated product of industrial, power generation, and weapons production activities has released large quantities of U and other waste products into subsurface environments, threatening water systems used for a wide variety of human activities. Accurate prediction of the fate and transport of U and other waste metals through complex, heterogeneous subsurface systems is essential.

The cell walls of the microbial communities present in well-populated, subsurface systems can represent a significant fraction of the total surface area exposed to fluids in groundwater systems. The study of U absorption onto bacteria, particularly at circumneutral pH conditions where U speciation is highly complex, is ongoing.

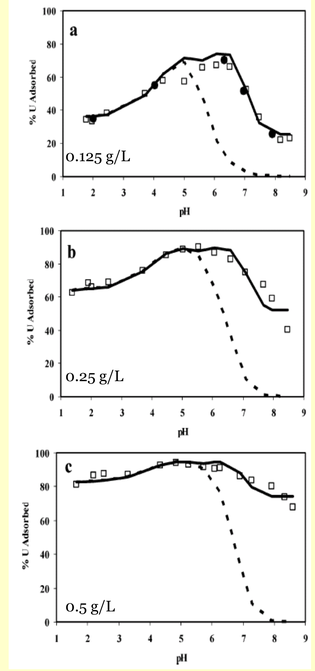
This study involves *B. subtilis*, a common groundwater bacterium. *B. subtilis* was cultured and prepared following the procedure of Fein et al. and involved a sequence of growth, rinsing, and centrifugation. From this wet mass, known bacterial concentrations were reacted with a U-bearing electrolyte solution. One series was exposed to the atmosphere (and thus in equilibrium with carbon dioxide), another was maintained in a closed atmosphere with carbon dioxide excluded. A final series was open to atmosphere and reacted with a Ca-bearing electrolyte solution. From these, five representative samples were chosen for XAS measurement.

Adsorbed U absorption was measured by ICP-OES. These absorption data were modeled as electrostatic interactions between the charged bacterial surface and the aqueous 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.



U absorbed by *B. subtilis* as a function of pH and bacterial concentration in a closed system (no CO_2). The curve is the best-fit surface complexation model, identifying 4 surface complexation sites.

U absorbed by *B. subtilis* as a function of pH and bacterial concentration in an open system (with CO_2). The solid curve is the best-fit surface complexation. The dashed curve is the predicted absorption in the absence of an inorganic uranyl carbonate surface complex.



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.

Restraint: 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 χ^2_ν.

$$\chi_\Gamma(k) = \frac{N_\Gamma S_0^2 F_\Gamma(k)}{2kR_\Gamma^2} e^{-2k^2\sigma_\Gamma} e^{-2R_\Gamma/\lambda(k)} \times \sin\left(2kR_\Gamma + \Phi_\Gamma(k) - 4k^3 C_{3,\Gamma}/3\right)$$

$$\chi_{\text{total}} = \sum_\Gamma \chi_\Gamma(k)$$

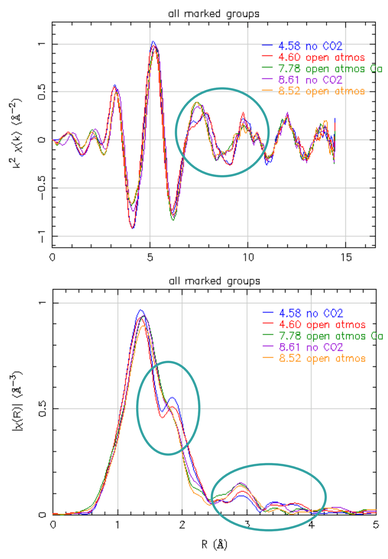
These constraints and restraints are applied to the terms of the EXAFS equation as math expressions using Ifeffit's built-in, infix math parser.

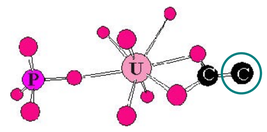
The parametric terms $(N_\Gamma, S_0^2, R_\Gamma, \sigma_\Gamma^2, E_0, C_{3,\Gamma})$ need not, themselves, be the variables in the fit. Rather, they are **expressed in terms** of the variables in the fit.

Five samples were measured:

1. closed system, no CO2, pH=8.61
2. closed system, no CO2, pH=4.58
3. open to atmosphere, pH=8.52
4. open to atmosphere, pH=4.60
5. open to atmosphere, pH=7.78, 10mM Ca added to bacterial suspension

0.5 g/L non-acid washed *B. Subtilis* in 0.1M NaClO4 with 1 ppm U. Samples were centrifuged and the paste placed in sample containers. All samples have approximately 400 ppm U on the solid.

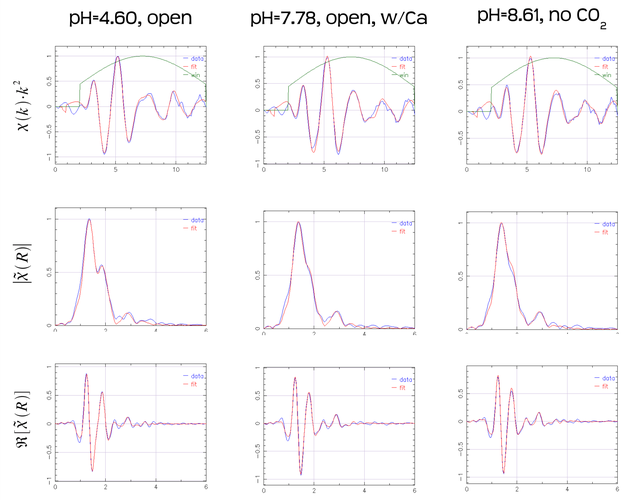




Material from the *B. subtilis* culture exposed to the U-bearing electrolyte solution were centrifuged to a paste and measured at the U L_{III} edge.

Low and high pH cultures were exposed to air or maintained in a CO_2-free atmosphere.

Data were measured by quick scan in transmission at the MRCAT beamline at the APS. Successive scans were merged to make the data shown to the left. The data fall into two groups distinguished by pH, with the differences marked by the turquoise circles.

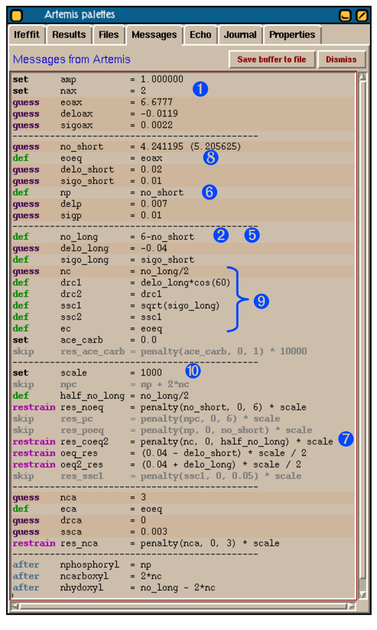


Fits were performed using theoretical standards from FEFF6 and the Artemis program (which uses Ifeffit as its back-end). The fitting model allows the equatorial oxygens to partition between phosphoryl, carboxyl/carbonato, and hydroxyl/hydration ligation. The best fit was obtained by also allowing sodium scatterers at about 4 Å.

The main distinction between the pH~4.6 samples and the higher pH samples was the number of phosphoryl ligands. Distances from U and the various scatterers (O, P, C) were consistent with values reported in the literature. Values for E0 and the various parameters were all reasonable.

Shown here are fits to three of the five samples. The remaining fits were of similar quality. In each case, the blue is the data, the red is the best fit function, and the green is the Fourier transform window. The fits were performed in *R*-space between 1 and 4 Å.

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 1.78Å.
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 about 6 equatorial oxygens, thus the value of 6 was asserted in the final fits.
3. Short equatorial oxygen atoms (R~2.27Å) are associated with phosphoryl complexation.
4. Long equatorial oxygen atoms (R~2.42Å) are associated with hydroxyl or carboxyl (or carbonato) complexation.
5. $N_{long} = 6 - N_{short}$
6. The number of phosphorous 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_0 is constrained to be the same for all paths. This constraint can be lifted by changing def to guess for `eoeq`, thus giving the axial oxygens an independent E_0.
9. The carboxyl/carbonato complex is treated as a rigid unit. `drc2` can be guessed to distinguish between carboxyl and carbonato ligation.
10. Restraints are scaled to a size that is relevant to the fit – a good rule of thumb is that the scaling factor should be about the size of χ^2 in the absence of the restraint.

Fitting results

pH	CO_2	Phosphoryl	Carbo(xyl|nato)	Hydroxyl
4.60	yes	2.13 (17)	1.18 (78)	2.69 (79)
4.58	no	2.36 (17)	1.19 (84)	2.45 (86)
7.78	yes	3.50 (19)	0.96 (86)	1.54 (88)
8.61	no	3.78 (25)	1.78 (1.24)	0.44 (1.26)
8.52	yes	3.44 (19)	0.59 (82)	1.96 (83)

Bond	length (Å)
axial oxygen	1.772 (4)
short equatorial	2.270 (9)
long equatorial	2.424 (8)
phosphorus	3.578 (16)
carbon	2.931 (36)
sodium	4.014 (28)

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 surface complex.

We do not see any indication of Ca in the Ca-bearing sample. Indeed, the fitting model for all samples used a Na 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.

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