

An EXAFS Study on the Biotransformation of Uranium by Halophilic and Non-Halophilic Bacteria

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Introduction: Transuranic (TRU) waste is disposed of in deep geological salt formations at the Waste Isolation Pilot Plant (WIPP), Carlsbad, New Mexico. However, there is limited knowledge of the mechanisms of biotransformation of TRU waste in a high ionic strength hypersaline environment. Microbes can affect the transport of actinides by forming biocolloids.^{1,2} Fundamental information on the mechanisms of microbial transformations of actinides under various environmental conditions is needed to predict the fate and long-term transport of actinides in waste repositories, as well as developing strategies for managing waste. In this study, we describe the biotransformation of uranyl nitrate by bacteria isolated from soils and from the hyper-saline WIPP repository.

Methods and Materials: A heterotrophic gram negative bacterium *Pseudomonas fluorescens* and an aerobic gram positive *Bacillus subtilis* were isolated from soils; and gram-negative halophiles *Haloanaerobium praevalens* and *Halobacterium halobium* were obtained from the WIPP repository.³ The cells were grown to early log phase in defined medium, and harvested by centrifugation. The resting cells were suspended in isotonic medium and exposed to uranyl nitrate at pH 5. The cells were placed in heat sealed polypropylene bags and the association of uranium with the bacterial cells was determined by EXAFS analysis at the LIII edge using fluorescence detection.

Results: The k^3 -weighted spectrum and corresponding Fourier transforms (-) and fitted data (--) for the bacteria are shown in **Figure 1**. Although the oscillations in the raw data are similar, there are differences in the broadness of the peaks at 7 and 10 \AA^{-1} (A). Uranium associated with *Halobacterium halobium* shows 2.0 axial oxygens at 1.77 \AA , 4.7 equatorial O's at 2.32 \AA , and 3.0 P atoms at 3.55 \AA . Uranium associated with *Haloanaerobium praevalens* shows 2.0 axial oxygens at 1.77 \AA , 4.2 equatorial O's at 2.32 \AA , and 4.0 P atoms at 3.55 \AA . Uranium associated with *Bacillus subtilis* shows 2.0 axial oxygens at 1.76 \AA , 4.6 equatorial O's at 2.32 \AA , and 2.0 P atoms at 3.53 \AA . Uranium associated with *Pseudomonas fluorescens* shows 2.0 axial oxygens at 1.77 \AA , 4.9 equatorial O's at 2.29 \AA , and 4.0 P atoms at 3.56 \AA . The number of U-P interactions was determined by restricting the Debye-Waller factor to that observed for the mixed standards. No bidentate carboxylate bonding of U to the cells was observed in any of the preparations. Additional ligands surrounding U not accounted for in the fit for the U-O equatorial region may include monodentate carboxylate oxygen, α -hydroxyl groups, and water, which are known to have distances at approx. 2.30 \AA .

Conclusions: Uranium was predominantly associated with the bacterial cells as uranyl phosphate. However, the presence of other functional groups is indicated for *B. subtilis* and *H. Halobium*. Additional studies (not shown) indicate the U is bound as phosphate to both intra- and inter-cellular components.

References: ¹A.J. Francis, J.B. Gillow, C.J. Dodge, M. Dunn, K. Mantione, B.A. Strietelmeier, M.E. Pansoy-Hjelvik, H.W. Papenguth, *Radiochim. Acta* **84**, 347 (1998); ²J.B. Gillow, M. Dunn, A.J. Francis, D.A. Lucero, H.W. Papenguth, *Radiochim. Acta*, in press; ³A.J. Francis, C.J. Dodge, J.B. Gillow, H.W. Papenguth, *Environ. Sci. Technol.* **34**, 2311 (2000).

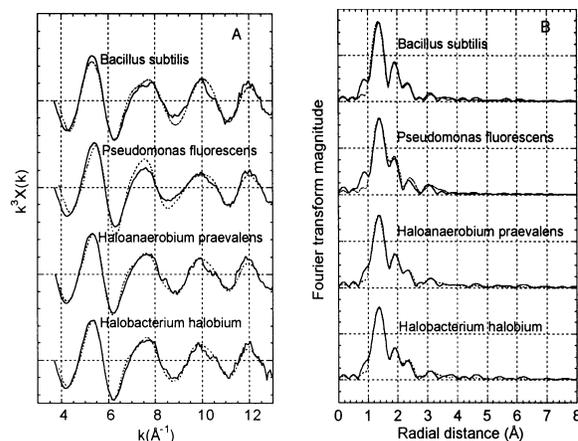


Figure 1. EXAFS analysis of U association with bacterial cells. Raw k^3 -weighted EXAFS data (A) and corresponding Fourier transforms (B) for halophilic and non-halophilic microorganisms.