# Toward an understanding of metal uptake in a methanogenic archaeon isolated from the Rancho La Brea Tar Pits

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Recalcitrant petroleum sources, such as heavy oil, oil sands, and asphalts, are an increasingly important part of the global petroleum supply. In situ conversion of these materials to methane would have significant environmental and economic advantages over current mining and extraction methods, but significant fundamental research on anaerobic methanogenic petroleum degradation is required before such approaches will become practical. Understanding the bioinorganic chemistry of such processes is essential to an overall understanding of methanogenesis, which requires a series of metallocofactors. Of these, several key proteins are nickel-binding proteins, including the hydrogenases. We are investigating mechanisms of nickel uptake in *Methanocorpusculum labreanum*, a methanogen isolated from natural asphalt sediments in the La Brea Tar Pits, Los Angeles. We have isolated a gene for a putative metalloregulatory protein from genomic DNA, cloned it into *E. coli*, and overexpressed and purified the resulting protein. Using a variety of spectroscopic methods, including UV-vis-NIR absorption, fluorescence, and circular dichroism spectroscopies, we are characterizing the coordination chemistry and structure of this protein. We have identified the presence of both flavins and iron-sulfur clusters, and work is currently underway to elucidate the structure and binding of these cofactors and the role they may play in regulating cellular nickel uptake.

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# Microbial uranium reduction monitoring: Linking isotopic fractionation factors with microbial metabolism

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Microbial reduction of soluble and mobile U(VI) to sparingly soluble U(IV) is a promising remediation strategy for uranium-contaminated aquifers. A wide range of microorganisms with diverse metabolic pathways can reduce U(VI) to U(IV). Microbial U(VI) reduction fractionates U isotopes; heavier isotopes partition in the solid U(IV) products. The remaining U(VI) pool becomes isotopically lighter with progressive reaction. The isotopic enrichment is quantified by measuring $^{238}U/^{235}U$ ratio in the remaining unreacted pool of U(VI) and the magnitude of the isotopic enrichment is expressed by the isotopic fractionation factor ε ($\varepsilon=1000*(\alpha-1)$; $\alpha = (^{238}U/^{235}U)_{Product}/(^{238}U/^{235}U)_{Reactant}$). The isotopic enrichment is related to the extent of reduction by the fractionation factor ε. $^{238}U/^{235}U$ ratios can be used to detect and possibly quantify U(VI) reduction, if the ε for the relevant microbial reductions are known.

Here we report ε values for U(VI) reduction by two strains of *Geobacter sulfurreducens* (PCA and Criddle), *Anaeromyxobacter dehalogenans* strain FRCW, *A. dehalogenans* strain FRC-R5, and *Desulfitobacterium* Viet1. For each bacterial species, we measured the ε values in duplicate batch incubation experiments with U(VI) and 0.5 mM electron-donor at 30 °C.

All strains tested in this study induced significant isotopic fractionation during U(VI) reduction. The ε yielded by *Geobacter sulfurreducens* strains PCA and Criddle were 0.68‰ and 0.95‰, respectively. The ε values for *A. dehalogenans* strain FRCW, *A. dehalogenans* strain FRC-R5, and *Desulfitobacterium* Viet1 were 0.75‰, 0.98‰, and 0.84‰, respectively. The results of this study indicate that the ε does vary with microbial metabolism. We observed an increasing trend in ε with decreasing cell-specific reduction rate. Our results suggest that cell-specific reduction rates are good indicators of the magnitude of microbial U isotopic fractionation. The ε tends to reach highest values (~1‰) under nutrient limited electron-donor poor conditions. Our results shed light on the fundamental relationship between metabolism and isotopic fractionation and will be useful to detect and possibly quantify U(VI) reductions in the sites undergoing active bioremediation.