Detoxification of Uranium under Sulfate-Reducing Conditions: Reactivity of Biologically Reduced Uraninite with Fe(III)-(hydr)oxides

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Uranium (U) is a known carcinogen, and the high solubility of its hexavalent form can result in U transport to sensitive receptors such as drinking water sources. One potential method of treating U contamination is by using natural dissimilatory metal-reducing bacteria such as sulfate-reducing bacteria (SRB) to reduce soluble U(VI) to insoluble U(IV) (as uraninite, UO_2), which can lead to in situ U immobilization and plume stabilization. Before SRB can be applied for bioremediation, however, it is important to understand U toxicity and the reactivity and stability of SRB-mediated uraninite. The effects of U(VI) on SRB were studied using *Desulfovibrio desulfuricans* G20 in a metal toxicity medium containing bicarbonate or PIPES pH buffer (each at 30 mM, pH 7). In batch experiments, U(VI) toxicity was dependent on the medium buffer and was observed in terms of longer lag times and, in some cases, no measurable growth. The minimum inhibiting concentration (MIC) that caused a complete inhibition of growth was 140 μM U(VI) in PIPES buffered medium. This is 36 times lower than previously reported for *D. desulfuricans*. The solubility of U(VI) was significantly lower in the PIPES buffer than in the bicarbonate buffer; however, U(VI) in the PIPES buffer was much more toxic than in the bicarbonate buffer. Analysis of thin sections of G20 treated with 90 μM U(VI) using HR-TEM revealed that reduced U was precipitated not only in the periplasmic spaces, but also in the cytoplasmic spaces. These results suggest that U(VI) toxicity and detoxification mechanisms of G20 greatly depend on the speciation of U(VI) present.

To examine the reactivity of reduced U in batch experiments, using *Desulfovibrio desulfuricans* G20, the effects of hematite, goethite, and ferrihydrite on the microbial reduction of U(VI) were evaluated under lactate-limited sulfate-reducing conditions. Our results suggested that once lactate was depleted, microbially reduced U served as an electron donor to reduce Fe(III) present in Fe(III)-(hydr)oxides. Subsequent addition of hematite [15 mmol Fe(III)/L] to stationary phase cultures containing microbially reduced U(IV) also resulted in rapid reoxidation to U(VI). Analysis by U L_3-edge XANES spectroscopy of microbially reduced U particles yielded spectra similar to those of natural uraninite. Observations by HR-TEM, SAED, and EDS analysis confirmed that precipitated U associated with cells was uraninite with particle diameters of 3–5 nm. These aqueous uraninite particles were separated from the bacterial cells using a 0.2 μm membrane filter, and treated with hematite and ferrihydrite. Both hematite and ferrihydrite oxidized uraninite; however, in these systems, the reactivity of uraninite was greater with ferrihydrite than with the more crystalline structure of hematite. The results have strong implications for field application of in situ biological reduction of U(VI). Care should be taken to maintain the organic substrate in excess throughout the system, such that the aquifer is electron-acceptor limited rather than electron-donor limited, at least until available Fe(III) is depleted from the system. Failing to maintain sufficient substrate concentrations until available Fe(III) is reduced could lead to unfavorable consequences for the long-term stability of immobilized uranium.