Investigation of the Transformation of Uranium under Fe\textsuperscript{III}-Reducing Conditions: Reduction of U(IV) by Biogenic Fe(II) in Green Rust

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**Biogenic Green Rust Formation Resulting from Dissimilatory Iron Reduction**

Defined mineral medium (Oma-Rigau et al., 2012 ES&T 36(1):16-20) containing 75 mM e-donor and 80 mM Fe(III) in sealed serum bottles was sparged with Ar and inoculated at an initial cell density of 5 X 10\textsuperscript{9} cell mL\textsuperscript{-1}. The suspensions were incubated at 30 °C in the dark. The reduction of Fe(III) was monitored by using the ferrozine assay to measure the Fe(II) content of 0.5 M HCl extracts of the suspensions. Biominalization products were identified by X-ray diffraction (XRD), Mössbauer spectroscopy, and scanning electron microscopy (SEM).

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**Reduction of Lepidocrocite by Shewanella spp.**

- **Analysis of the solids remaining after 57 d by SEM, Mössbauer spectroscopy and XRD indicated that the lepidocrocite was completely transformed by S. putrefaciens CN32, with carbonate green rust as the only significant solid-phase Fe(II)-bearing product. Carbonate green rust was also the only Fe(II) solid phase formed from lepidocrocite reduction by the other Shewanella spp. examined (with the exception of S. denitrificans OS2117).**

**e Donor Effects on Reduction of \(\gamma\)-Lepidocrocite by S. putrefaciens CN32**

Of the e-donors tested, formate, \(\text{H}_2\), lactate, \(\text{N-acetylglucosamine}\), pyruvate, and serine supported significant Fe(II) reduction. Fe(II) production in the absence of added e-donor is attributed to utilization of endogenous e-donor(s).

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**Reduction of U(IV) by Biogenic Green Rust**

**Preparation of Biogenic Green Rust**

Biogenic green rusts were prepared by the reduction of lepidocrocite by S. putrefaciens CN32, S. alga BY1, and S. sp. ANA-3 as described in the panel to the left. The biogenic green rust suspensions (containing ~60 mM Fe(II)) were pasteurized at 70 °C for 1 h; this treatment was sufficient to kill the Shewanella spp., with minimal alteration of the biogenic green rust. The pasteurized biogenic green rust was then repeatedly sonicated and washed with DI water. The initial pH of these suspensions ranged from 7.3 – 7.5.

**U(IV) Interaction with Biogenic Green Ruts**

The biogenic green rust suspensions were spiked with a stock solution of uranyl chloride. The resulting suspensions had initial U(IV) and Fe(II) concentrations of 625 µM and 60 mM, respectively. After 48 h, the pH of the suspensions was measured, the suspensions were centrifuged, and the supernatants were saved for uranium analysis by inductively coupled plasma-optical emission spectroscopy (ICP-OES). Uranyl was readily removed from solution in the presence of biogenic green rust. Within 48 h, solution-phase uranium concentrations decreased from 625 µM to 1.6 µM. The final pH of the suspensions ranged from 5.8 – 6.0.

**EXAFS Analysis of U associated with Biogenic Green Rust**

The solids remaining after centrifugation (hereafter designated as UBioGR) were analyzed by extended X-ray absorption fine structure (EXAFS) spectroscopy. Measurements at the U L\textsubscript{3} absorption edge (17164 eV) were performed at the Materials Research Collaborative Access Team (MRCAT) beamline 10-ID at the Advanced Photon Source (APS) at Argonne National Laboratory. Energy scans were collected at different locations on the sample to reduce radiation exposure; however, no time-dependent change in the data was observed for any of the samples.

The EXAFS data were fit with a model based on the structure of uraninite (UO\textsubscript{2}\textsubscript{3}), but modified to include contributions from 1-3 Fe atoms and a splitting of the O1 shell (O1a, O1b, O1c). The EXAFS results for UGRCl and UGRSO\textsubscript{4} are compared to the EXAFS results for UGRCO\textsubscript{3} and UGRSO\textsubscript{4} data in the table below.

**Fit Results**

Fit results for the EXAFS data obtained from UBioGR samples. The average number of Fe atoms is consistent with 1 – 3 Fe neighbors per U atom. The U(IV) appears to be present as small uraninite clusters containing 2 – 6 U atoms on average.

**EXAFS Data**

EXAFS data (symbols) of UBioGR samples with the corresponding model fits indicated by the solid lines. The fit range was 3.5 – 7.5 Å.

**Model Components**

- The magnitude (A) and real part (R) of the Fourier transform of the EXAFS data (symbols) from the S. sp. ANA-3 UBioGR sample and the model fit (top blue curve).
- The components of the model corresponding to the various coordinating atoms are shown by the curves below the EXAFS data.

**Fourier Transforms of UBioGR Data**

The magnitude (A) and real part (R) of the Fourier transform of the EXAFS data (symbols) from the S. alga BY1, S. sp. ANA-3, and S. putrefaciens CN32 UBioGR samples and the model fit (lines).

The results of the EXAFS data analysis indicate that U(IV) is reduced to U(VI) by biogenic green rusts produced by several Shewanella. In these systems the U(IV) is present as small molecular clusters with a structure similar to uraninite. These clusters appear to be associated with Fe.

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**Interlayer Anion Effects on the Reduction of U(VI) by Green Rust**

**Green Rust Synthesis**

Green rusts were synthesized by precipitation of a ferrous/ferric mixture, with appropriate anions, by the addition of NaOH (sulfate, chloride) or Na\textsubscript{2}CO\textsubscript{3} (carbonate) to pH 8. The green rust precipitate was filtered and freeze dried under anaerobic conditions to produce a fine blue-green powder that was confirmed to be green rust by x-ray diffraction.

**Kinetics of U(VI) Removal**

Kinetic data were collected from batch reactors containing sulfate, chloride, or carbonate green rust at a solids loading of 3 g L\textsuperscript{-1} and a solution of 400 µM U(VI) in 0.1 M TAPS buffer at pH 8.0. Samples were collected throughout the experiment and passed through a 0.2 µm filter. Aqueous U(VI) concentrations were measured by ion chromatography (DeBeer and Coetzee, 1992, Radiochim. Acta. 57:113-117).

**Removal of U(VI) from solution**

Although there are differences in the extent of uptake, > 90% of the removal of U(VI) from solution occurred within 50 min for each of the green rusts examined.

**U(VI) Adsorption or Reduction?**

To determine whether U(VI) is adsorbed or reduced by the green rusts, XAFS spectroscopy was used to distinguish U(VI) from U(VI) in the product as well as provide information about the coordination environment of the uranium atom.

**XANES spectra for UGRCl, UGRSO\textsubscript{4}, and UGRSO\textsubscript{4} and for U(VI) and U(VI) standards**

A comparison between the U-XANES spectra of the U(VI) and U(VI) standards and UGRCl, UGRSO\textsubscript{4}, and UGRSO\textsubscript{4} clearly shows that the U(VI) added to GRCl and GRSO\textsubscript{4} suspensions is reduced to U(VI). Conversely, there was no indication of U(VI) reduction to U(VI) in GRCO\textsubscript{3} suspensions.

**EXAFS results for UGRSO\textsubscript{4}, UGRCl, and UGRSO\textsubscript{4}**

The peak representing the U-peak in the Fourier transformed EXAFS data for U(II) is highly attenuated in the data for GRCl and GRSO\textsubscript{4} which is consistent with molecular clusters of UO\textsubscript{2} and a splitting of the O1 shell. Peaks corresponding to the equatorial and axial oxygen atoms in uranyle are evident in the data for GRCO\textsubscript{3}.

In GRCl and GRSO\textsubscript{4} suspensions > 99% of added U(VI) is removed from solution with > 90% reduced to U(VI). The EXAFS data for the resulting U(VI) phase are consistent with the formation of single molecules or small molecular clusters of UO\textsubscript{2}. Although > 80% of U added to the GRCO\textsubscript{3} suspensions is associated with the solid phase, there is no indication of significant (> 10%) reduction to U(VI). These results clearly indicate that the reactivity of green rusts with respect to the reduction of U(VI) to U(VI) is affected by the nature of the interlayer anion.

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