

## Supporting Information

# Influence of iron sulfides on abiotic oxidation of UO<sub>2</sub> by nitrite and dissolved oxygen in natural sediments

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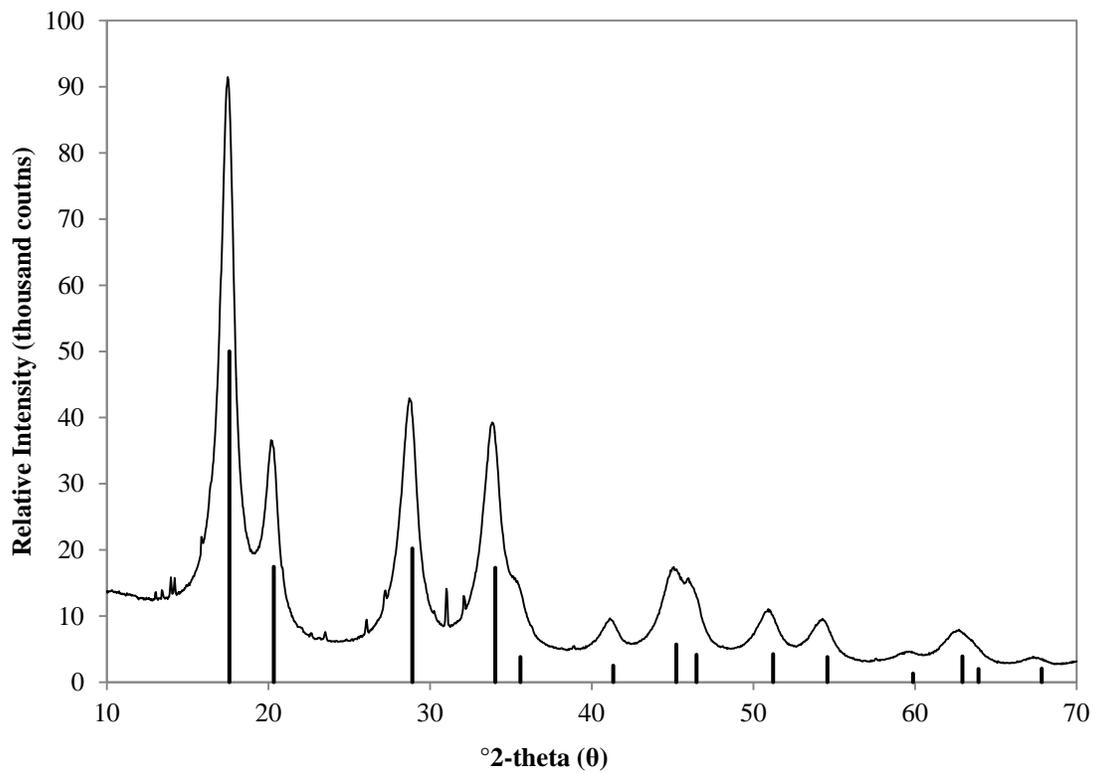
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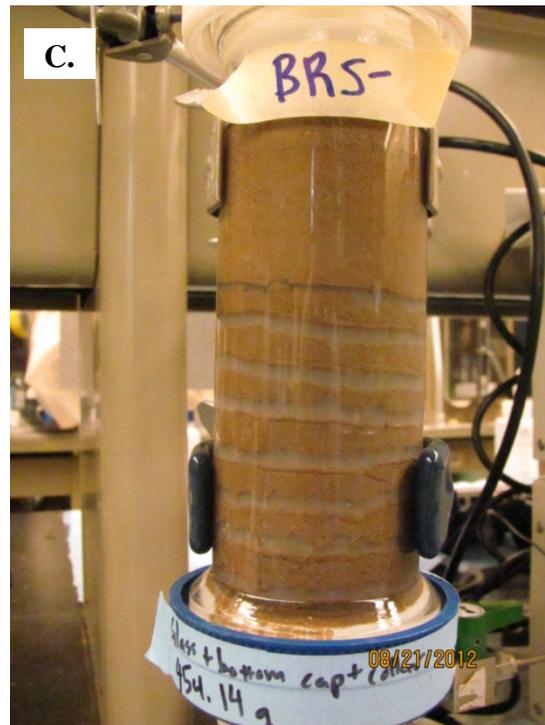
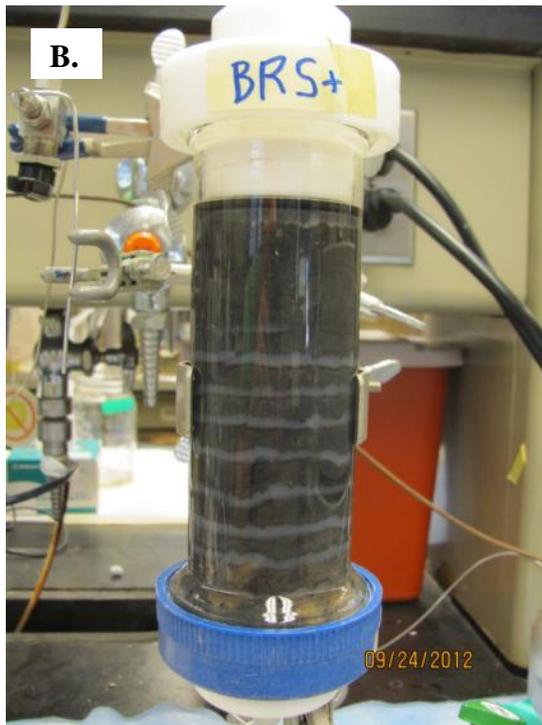
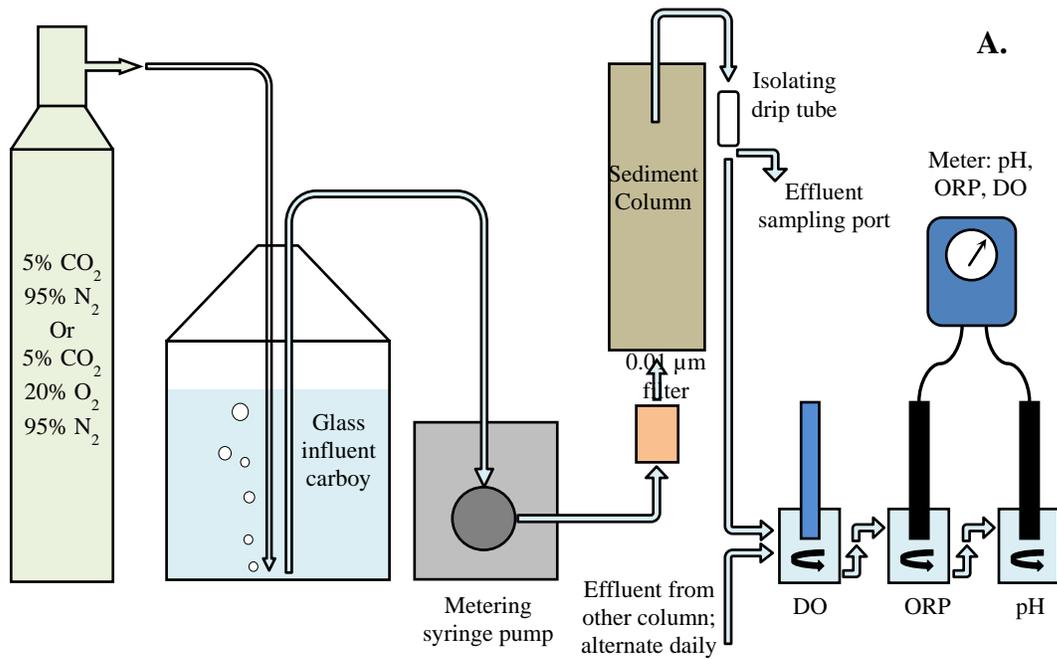
This Supporting Information covers 10 pages, including 3 sections, 5 figures, and 1 table.

## A. Synthetic uraninite characterization



**Figure S1.** Synchrotron X-ray diffractogram of synthetic uraninite. Substantial peak broadening indicates nano-scale crystallite size.

## B. Column flow schematic



**Figure S2.** (A) The schematic of the flow-through reactor system. (B) The BRS+ column after 62 days of bioreduction under sulfate reducing conditions; (C) the BRS- column after 62 days of bioreduction in the absence of sulfate.

### **C. Identification of biogenic mackinawite in reduced sediment**

Least-squares linear combination fitting (LCF) was used to determine the dominant sulfur species in BRS+ bioreduced sediment (a multiple-component mixture) from their fingerprint in the XANES region. A number of sulfur reference compounds were used, including biogenic mackinawite produced by *Desulfovibrio vulgaris*, slightly oxidized biogenic mackinawite, synthetic mackinawite, synthetic greigite, pyrite, sulfate, thiosulfate, and AR-grade S<sub>8</sub><sup>0</sup>.

#### *a. Biogenic mackinawite solid preparation*

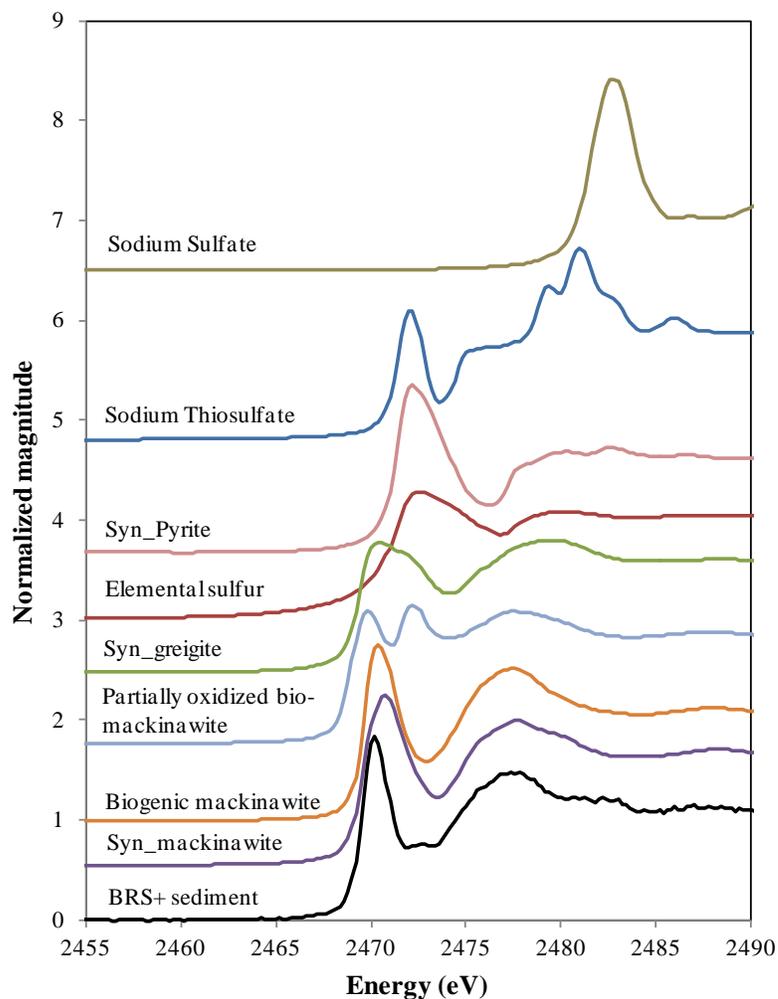
Biogenic mackinawite was produced via microbial sulfate reduction according to the methods outlined in Zhou et al. (1). Briefly, *Desulfovibrio vulgaris* strain was cultured and grown in ATCC medium, which contained dissolved sulfate and ferrous iron. After 7 days of incubation, black precipitates were separated from the supernatant by letting the reaction mixture settle overnight in the glove box. Once a clear supernatant was formed, the solids were separated by decanting the supernatant. Iron sulfide solids were then washed with sterile anaerobic 30 mM NaHCO<sub>3</sub> solution and DI water to remove any remaining growth medium and biomass debris. The solids were stored in serum bottles inside an anaerobic chamber (5% H<sub>2</sub>, 95% N<sub>2</sub>) until use. Partially oxidized biogenic mackinawite was prepared by exposing a serum bottle containing biogenic solids to the air for 1 hour.

#### *b. Abiotic sulfur species preparation*

Synthetic mackinawite was prepared following the procedure described in Butler et al (2). The material was characterized to be nanocrystalline mackinawite with structural properties similar to a previous study (3).

A synthetic greigite sample was synthesized basically following a hydrothermal method described in Chang et al. (4). Briefly, ferric chloride ( $\text{FeCl}_3$ ) and thiourea ( $\text{CH}_4\text{N}_2\text{S}$ ) are reacted under acidic (achieved by formic acid [ $\text{HCOOH}$ ]) and anaerobic conditions with asserted high pressure and temperature. A black precipitate was obtained after the hydrothermal reaction by decanting the solution and washing the precipitate with carbon disulfide to remove residual elemental sulfur. The washed black particles then were moved into the anaerobic chamber and repeatedly rinsed with deoxygenated Milli-Q water and pure ethanol. The final solids were freeze dried and stored in a serum bottle until XANES analysis.

Pyrite was purchased from Alfa Aesar as naturally occurring mineral (CAS 1309-36-0). Sulfate and thiosulfate were sodium sulfate and sodium thiosulfate salt, respectively. AR-grade  $\text{S}_8^0$  powder (99.98%) were purchased from Sigma-Aldrich (CAS 7704-34-9). All solids were ground to fine particles with mortar and pestle before XANES analysis.

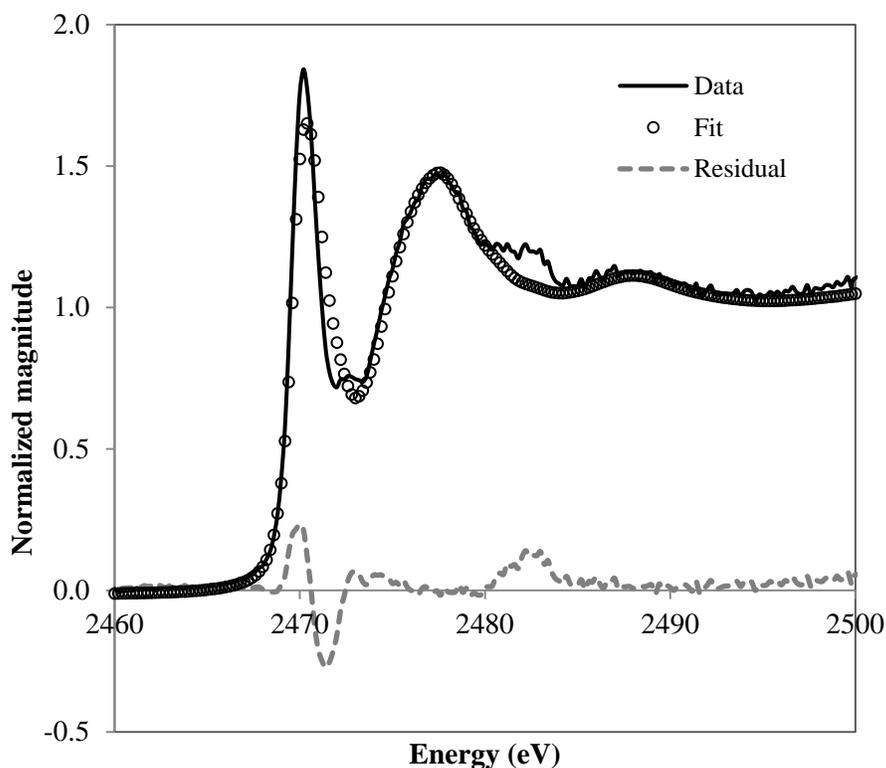


**Figure S3.** Sulfur K-edge XANES spectra of all reference standards used in the LCF analysis, including biogenic mackinawite produced by *Desulfovibrio vulgaris*, slightly oxidized biogenic mackinawite, synthetic mackinawite, synthetic greigite, pyrite, sodium sulfate, sodium thiosulfate, and AR-grade  $S_8^0$ .

*c. LCF analysis for dominant sulfur species*

The many standard compounds as described above were measured and fitted by different combinations to the sediment data. The fits consider all possible binary, ternary, and quaternary combinations of standards. The best fitting result (Figure S3) for the BRS+

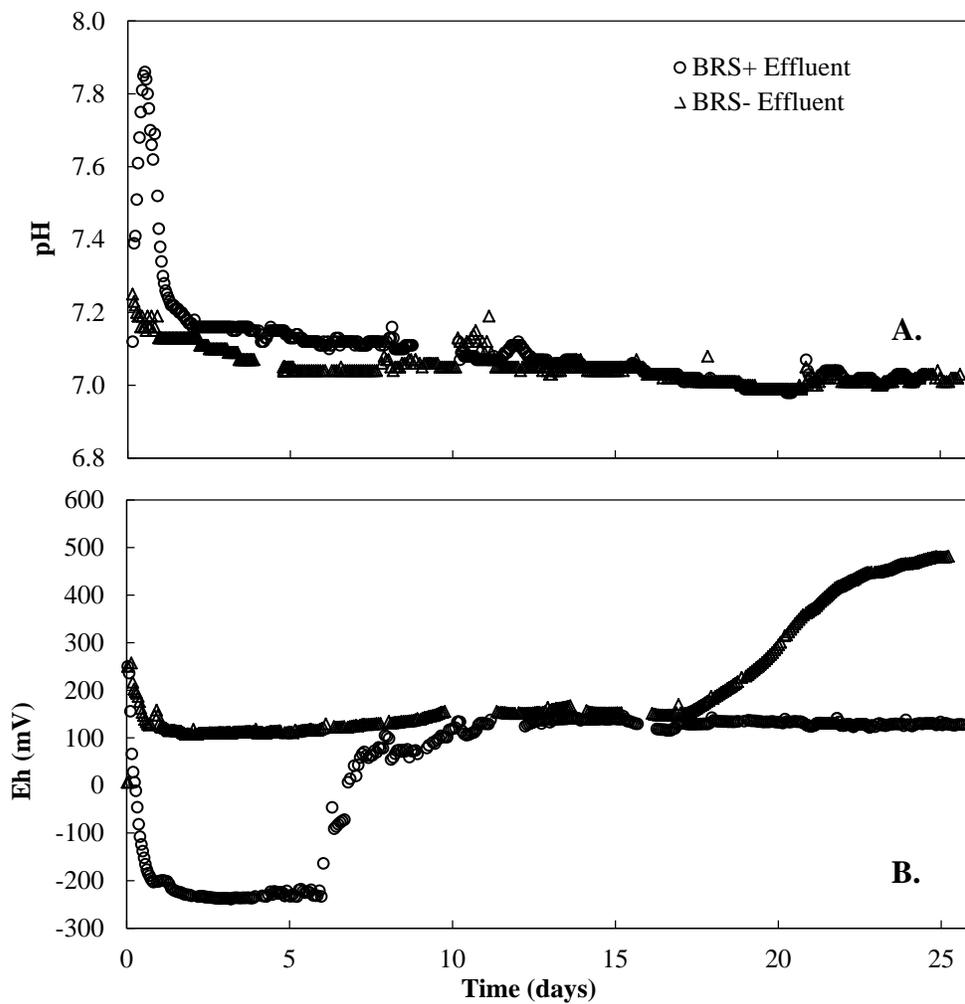
sample suggests that the sulfur species consisted predominantly of biogenic mackinawite (~ 83%). The remaining sulfur was fitted by the partially oxidized biogenic mackinawite reference compound (<17% of total sulfur) (Table S1). Compared to biogenic mackinawite, the synthetic counterpart was not considered representative of the dominant sulfur phase. In addition, synthetic greigite, pyrite, sulfate, thiosulfate, and AR-grade  $S_8^0$  were not included in the fit given that their spectra were greatly different from that of the sediment and mackinawite. Although a low R-factor value (0.00373) suggests a good fit for the sediment sample, the fitting residual imply that other minor reduced sulfur constituents may also be present in sediment column. Alternatively, the biogenic mackinawite standard used for LCF fitting may not perfectly represent the iron sulfide phase generated in the sediment. Nevertheless, forms of mackinawite best describe the sulfur species present in the column under sulfate-reducing conditions, as reported in previous sediment column studies (5, 6).



**Figure S4.** The result of best fit to the BRS+ column XANES spectra from a combinatorial sequence. The included standard compounds are biogenic mackinawite, partially oxidized biogenic mackinawite, and synthetic mackinawite.

**Table S1.** Sulfur speciation, expressed as % of total sulfur, for the bioreduced sediment sample based on the calculation of *K-edge* XANES linear combination fitting.

	<b>Biogenic mackinawite</b>	<b>Partially oxidized biogenic mackinawite</b>	<b>Synthetic mackinawite</b>	<b>Synthetic Greigite</b>
Sediment	82.9 (3)	17.1 (2)	0 (0)	0 (0)
<i>R-factor:</i>	<i>0.00373</i>	<i>Reduced chi square:</i>	<i>0.00313</i>	



**Figure S5.** (A) Effluent pH during abiotic oxidation phase in BRS+ and BRS- columns; (B) Effluent Eh (mV) over the course of the oxidation phase for both columns. Nitrite (0.53 mM) was introduced at day 6 and was replaced by dissolved oxygen (0.25 mM) on day 15.5.

## Reference:

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