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### **Title**

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1 **Real-Time X-ray Absorption Spectroscopy of Uranium, Iron, and Manganese in**  
2 **Contaminated Sediments During Bioreduction**

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10

11 **Abstract**

12 The oxidation status of uranium in sediments is important because the solubility of this toxic and  
13 radioactive element is much greater for U(VI) than for U(IV) species. Thus, redox manipulation  
14 to promote precipitation of UO<sub>2</sub> is receiving interest as a method to remediate U-contaminated  
15 sediments. Presence of Fe and Mn oxides in sediments at much higher concentrations than U  
16 requires understanding of their redox status as well. This study was conducted to determine  
17 changes in oxidation states of U, Fe, and Mn in U-contaminated sediments from Oak Ridge  
18 National Laboratory. Oxidation states of these elements were measured in real-time and  
19 nondestructively using X-ray absorption spectroscopy, on sediment columns supplied with  
20 synthetic groundwater containing organic carbon (OC, 0, 3, 10, 30 and 100 mM OC as lactate)  
21 for over 400 days. In sediments supplied with OC ≥ 30 mM, 80% of the U was reduced to U(IV),  
22 with transient reoxidation at about 150 days. Mn(III,IV) oxides were completely reduced to  
23 Mn(II) in sediments infused with OC ≥ 3 mM. However, Fe remained largely unreduced in all

24 sediment columns, showing that Fe(III) can persist as an electron acceptor in reducing sediments  
25 over long times. This result in combination with the complete reduction of all other potential  
26 electron acceptors supports the hypothesis that the reactive Fe(III) fraction was responsible for  
27 reoxidizing U(IV).

28

## 29 **Introduction**

30 The mobility of U through sediments is strongly influenced by its oxidation state (*1*).  
31 Reduction of U(VI) to less soluble U(IV) occurs through metal (primarily Fe) and sulfate  
32 reducing microorganisms (2-4), and through abiotic redox reactions with Fe(II) (5), green rusts  
33 (6), and S(-II) (7). Conversely, oxidation of U(IV) to U(VI) occurs rapidly in the presence of  
34 oxygen (8, 9), nitrate/nitrite (*10, 11*), Mn(III,IV) (12), and some Fe(III) (hydr)oxides (13-16).  
35 The behavior of U in sediments during the transition from oxidizing to reducing conditions is  
36 complicated because of coupling between numerous reactions, with transformations progressing  
37 at widely varying rates.

38 In our previous study on historically U-contaminated Oak Ridge National Laboratory  
39 sediments, U(IV) reoxidation occurred under sustained reducing conditions (*14, 17*). Uranium-  
40 contaminated sediments were infused with a steady supply of organic carbon (OC, 10.7 mM  
41 lactate = 32 mM OC, at an OC supply rate of 0.55 mmol kg<sup>-1</sup> d<sup>-1</sup>). Changes in U oxidation states  
42 were determined nondestructively within columns using micro- X-ray absorption near edge  
43 structure ( $\mu$ -XANES) spectroscopy. Rapid U(VI) reduction during the initial 80 days was  
44 followed by reoxidation of U(IV) and increased U(VI) concentrations in effluents. Reoxidation  
45 of U(IV) was unexpected because these columns were maintained under continuously reducing  
46 conditions. We found that U(VI) enrichment in effluents resulted from complexation with

47 carbonates produced by microbial OC oxidation. Reactive Fe(III) and/or Mn(IV) oxides were  
48 suspected as the terminal electron acceptors (TEAs) for U(IV) reoxidation, but that earlier  
49 experimental system did not permit direct, nondestructive speciation of Fe and Mn.

50 This present study follows the course of the previous work, with the main goal of testing  
51 the hypothesis that Fe(III) and Mn(III,IV) oxides persist long enough during U bioreduction to  
52 permit U reoxidation under sustained metal-reducing conditions. The experiments involved  
53 infusion of synthetic groundwater containing different concentrations of OC into U-contaminated  
54 sediments, and periodically obtaining  $\mu$ -XANES spectra of U, Mn, and Fe to directly monitor  
55 oxidation states of each element. In this paper, OC does not refer to native sediment organic  
56 matter unless indicated. The study presented here is part of a larger effort designed to  
57 understand impacts of different OC forms and supply rates on U transformations and on  
58 microbial communities.

59

## 60 **Materials and Methods**

61 **Sediment.** Uranium-contaminated sediment was obtained from the U.S. Department of Energy's  
62 Environmental Remediation Science Program's Field Research Center (FRC) at Oak Ridge  
63 National Laboratory. The sediment had a total U concentration of  $1.08 \text{ mmol kg}^{-1}$  (X-ray  
64 fluorescence analysis, XRF), and was from a location near that of our previous study (14). The  
65 total Mn and Fe in this sediment were  $49.1$  and  $1,185 \text{ mmol kg}^{-1}$ , respectively (XRF). Citrate-  
66 dithionite method (18) yielded an extractible Fe concentration of  $248 \text{ mmol kg}^{-1}$ . A  $0.5 \text{ M HCl}$   
67 extraction paired with a  $0.25 \text{ M HCl/hydroxylamine hydrochloride}$  extraction (19) yielded an  
68 extractible Fe(II) concentration of  $0.23 \text{ mmol kg}^{-1}$ , and operationally defined microbially  
69 reducible Fe(III) concentration of  $5.9 \text{ mmol kg}^{-1}$ . This sediment had a native OC concentration of

70 370 mmol kg<sup>-1</sup> (0.44%). Additional geochemical analyses of the sediment are provided in  
71 Supporting Information Table S1. Moist sediment was passed through a 4.75 mm sieve and  
72 homogenized prior to packing into columns to a porosity of 0.51.

73 **Sediment columns.** 200 mm long, 31.5 mm inner diameter polycarbonate columns were similar  
74 to those used previously (14), except that thinner windows were installed to permit sufficient X-  
75 ray penetration into sediments and fluorescent X-ray collection at the lower energies of Mn and  
76 Fe K absorption edges. In order to maintain the structural integrity of each column, the windows  
77 were made in 2 sections (70 mm by 10 mm) separated by a solid pipe segment (Supporting  
78 Information, Figure S1). Two layers of Kapton tape were use for each window, such that the  
79 adhesive surfaces were sandwiched between Kapton films in order to prevent redox reactions  
80 between the sediment and adhesive (20). Kapton windows were secured on columns with  
81 aluminum frames (0.60 mm thick), and caulked with epoxy glue. Windows were covered with  
82 plastic plates banded to the column in order to limit slow expansion of the sediment against the  
83 thin Kapton film, and uncovered during  $\mu$ -XANES data collection. Platinum redox electrodes  
84 were embedded into each column at distances of 50, 100, and 150 mm.

85 **Influent solutions.** A synthetic groundwater solution was prepared based on the composition of  
86 an uncontaminated groundwater from the Oak Ridge FRC. Its major ion chemistry consisted of  
87 0.83 mM Ca<sup>2+</sup>, 0.20 mM Mg<sup>2+</sup>, 2.00 mM Na<sup>+</sup>, 0.10 mM K<sup>+</sup>, 2.1 mM Cl<sup>-</sup>, 1.00 mM HCO<sub>3</sub><sup>-</sup>, 0.50  
88 mM SO<sub>4</sub><sup>2-</sup>, and 0.05 mM NO<sub>3</sub><sup>-</sup>, had an ionic strength of 5.68 mM, pCO<sub>2</sub>  $\approx$ 3.5, and pH = 7.3.  
89 Low levels of NO<sub>3</sub><sup>-</sup> and SO<sub>4</sub><sup>2-</sup> were included in these solutions because of their natural  
90 occurrence in groundwater, hence their likely inclusion in remediation relying on injection of  
91 OC-amended local groundwater. For the OC supply at different concentrations, Na-lactate was

92 added to the synthetic groundwater to obtain solutions containing 0, 3, 10, 30, and 100 mM OC,  
93 with all solutions autoclaved.

94 **Sediment column operation.** Columns were kept in an N<sub>2</sub>-purged glovebox except during  
95 times for transporting to synchrotron facilities for  $\mu$ -XANES data collection. Solutions were  
96 supplied via syringe pumps at an average pore water velocity of 8.2 mm day<sup>-1</sup> (24 day residence  
97 time). After supplying columns with 2 pore volumes (1 PV = 79.5 mL) of the 0 mM OC  
98 solution, infusion with different OC concentration solutions (including continuation of one  
99 column with 0 mM OC) was initiated. The combination of influent OC concentrations and pump  
100 rate yielded column-averaged supply rates ranging from 0 to 1.4 mmol OC kg<sup>-1</sup> day<sup>-1</sup>. Effluents  
101 were collected using a fraction collector. Redox potentials were periodically obtained by Pt  
102 electrode readings referenced to a calomel electrode tapped into the outflow end of each column.  
103 When columns were to be transported to synchrotron facilities, endcap valves were shut and  
104 disconnected from inflow/outflow lines, and columns were sealed in individual N<sub>2</sub>-purged plastic  
105 bags.

106 **Micro-XANES spectroscopy.** Oxidation states of U, Fe, and Mn in sediments were obtained  
107 using  $\mu$ -XANES spectroscopy (21, 22). The X-ray absorption spectra of each of these elements  
108 were obtained at the GeoSoilEnviroCARS beamline 13ID-C at the Advanced Photon Source  
109 (APS, Argonne National Laboratory, Argonne, IL), and at beamline X26A of the National  
110 Synchrotron Light Source (NSLS, Brookhaven National Laboratory, Upton, NY). At both  
111 facilities, beam sizes of  $\approx 200$   $\mu$ m (vertical) by  $\approx 800$   $\mu$ m (horizontal) were used in order to  
112 average over a large population of mineral grains and pores. The broad beam size also helped  
113 minimize X-ray beam-induced reduction (23). Profiles of  $\mu$ -XANES spectra were obtained

114 along each sediment column, sequentially for Mn, Fe and U, at 7 different times during the  
115 course of this study.

116 Oxidation state standards for Mn consisted of  $\text{MnCO}_3$  for defining the Mn(II) edge  
117 energy, Na-birnessite,  $\text{MnO}_2$  for defining the Mn(IV) edge energy, and  $\text{KMnO}_4$  for energy  
118 calibration, each mixed into  $\text{SiO}_2$  powder to about 1% Mn. A Mn(III) standard was not included  
119 because of the priority placed on determining redox status within the range set by Mn(IV) and  
120 Mn(II), and the interest in avoiding radiation-induced redox changes. Resolution of all 3 Mn  
121 oxidation states would require longer X-ray exposures for collection of  $\mu$ -XANES data at high  
122 resolution. The Mn(VII) pre-edge peak energy was set equal to 6,543.3 eV (24). Examples of  
123 these Mn K-edge spectra are shown in Figure 1a. Because several different energy steps were  
124 taken within individual scans, spectra were smoothed piecewise using an extension of the  
125 Savitzky-Golay method developed by Gorry (25). This smoothing was also applied to the Fe and  
126 U spectra. Manganese K-edge scans on soils were obtained with coarse steps in the below edge  
127 region (6,530 to 6540 eV, in 2 eV step), finer steps in the edge region (6541 to 6565 eV, in 0.5  
128 eV steps), and coarse steps above the absorption edge (6,630 to 6,650 eV, in 5 eV steps) for  
129 normalizing the step height. The Mn  $\mu$ -XANES measurements on sediment columns were done  
130 with high resolution only along the main absorption edge in order to minimize the potential for  
131 X-ray beam induced redox changes. Local Mn redox status was characterized by determining  
132 the energy along the absorption edge corresponding to half the absorption of the step height (26),  
133 then comparing this half-height energy to those of Mn(II) and Mn(IV) standards. These edge  
134 energies differ by  $6.0 \pm 0.5$  eV.

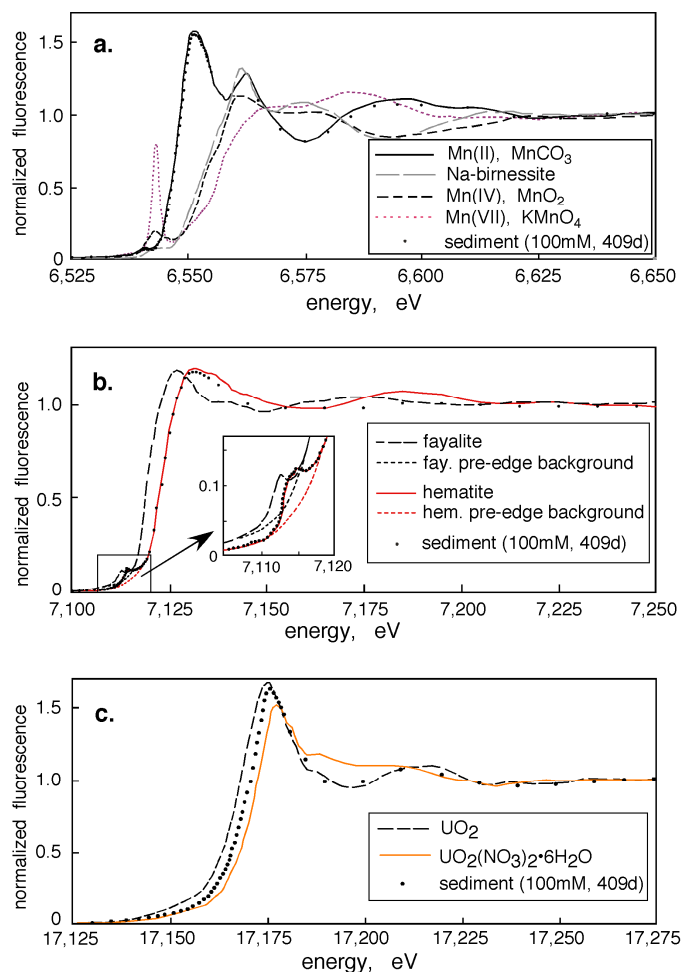
135 Iron oxidation states were characterized using the energy of the 1s-3d pre-edge centroid,  
136 which shifts about -2.5 eV in going from Fe(III) to Fe(II) (27-29). Oxidation state standards

137 used were hematite and goethite for Fe(III), and fayalite for Fe(II), with their spectra shown in  
138 Figure 1b. An Fe foil was used for energy calibration, with its edge energy taken as 7,112.0 eV.  
139 With this calibration, the pre-edge centroid energies for hematite and goethite standards were  
140 7,114.9  $\pm$ 0.1 eV. Distinguishing among different Fe(III) oxides within sediments by XANES  
141 spectroscopy is challenging (30), and was not attempted. Iron K-edge spectra were collected  
142 over energy ranges starting at or below 7,050 eV and finishing at 7,300 eV or higher, with 0.1 eV  
143 steps in the pre-edge region (7,110 to 7,120 eV), and coarser (0.25 to 2 eV) steps elsewhere.

144         Oxidation states for U in sediments were obtained through determining U L<sub>III</sub> absorption  
145 edges, specifically through the edge energies at half that of the step height (31). Monochromatic  
146 X-ray energies were scanned from about -40 to +200 eV relative to the U L<sub>III</sub> absorption edge,  
147 using 0.2 eV steps within the main edge, and coarse (2 to 5 eV) steps over the pre-edge and edge  
148 step regions. The U(VI) and U(IV) oxidation states were assigned half step height energies of  
149 17,168.0 and 17,163.7 eV, using UO<sub>2</sub>(NO<sub>3</sub>)<sub>2</sub> and UO<sub>2</sub> (both diluted in SiO<sub>2</sub> powder),  
150 respectively (Figure 1c). Monochromator calibration at this high-energy region was done with Y  
151 foil, with its absorption edge taken as 17,038.0 eV.

152





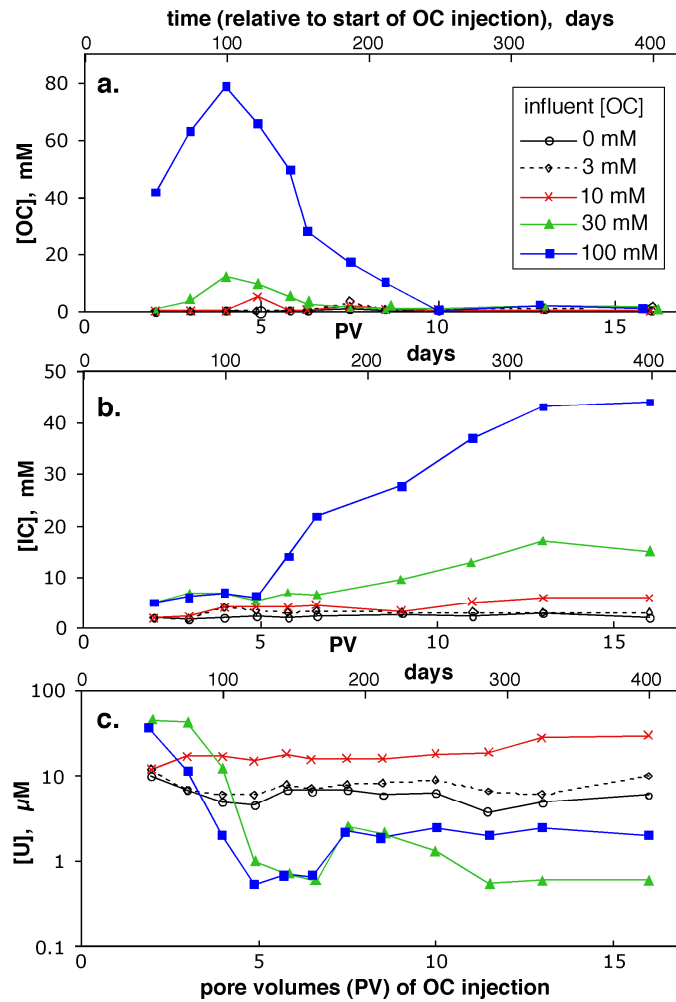
153  
 154 **Figure 1.** XANES spectra of oxidation state standards (a.) Mn K-edges, (b.) Fe K-edges with  
 155 insert close-up view of pre-edge peak region, and (c.) U L<sub>III</sub> edges. Examples of spectra  
 156 collected from the 100 mM sediment column are also included.

157  
 158 **Results and Discussion**

159 While this paper is focused on changes in oxidation states of Mn, Fe, and U within sediments,  
 160 aspects of the effluent solution chemistry important for understanding redox transformations are  
 161 summarized in Figure 2 and Table S2. The main microbiological characteristics follow those  
 162 presented in our earlier study of U bioreduction (14, 17). Most of the lactate supplied to columns  
 163 was mineralized, such that bicarbonate levels in effluents were elevated in proportion to influent

164 OC concentrations (Figure 2a,b). Uranium concentrations rapidly decreased for columns  
165 supplied with OC  $\geq$  30 mM, followed by increased U concentrations resulting from formation of  
166 soluble U(VI) tricarbonates and dicalcium uranyl tricarbonates (14), and reached steady  
167 values in the range of 0.5 to 30  $\mu$ M after about 200 days (Figure 2c). The intermediate OC  
168 supply rate of 0.14 mmol kg<sup>-1</sup> day<sup>-1</sup> (10 mM OC influent) resulted in the highest effluent U  
169 concentrations because of enhanced U(VI) solubility from carbonate complexes while achieving  
170 only minor U reduction. The low levels of nitrate (50  $\mu$ M) in influent solutions were reduced  
171 below detection (0.3  $\mu$ M nitrate and nitrite) in effluents of all OC-supplied columns, and nearly  
172 completely reduced ( $2.4 \pm 2.4$   $\mu$ M in effluents) in the column that received no OC. Nitrate and  
173 nitrite have been shown to promote U(IV) reoxidation by directly serving as a TEA and through  
174 oxidizing Fe(II) to Fe(III), which in turn oxidizes U(IV) (10, 15, 32). Sulfate reduction was  
175 proportional to the OC supply, with total S levels in effluents falling below 5  $\mu$ M in the 30 and  
176 100 mM OC columns (Table S2).

177



178

179

180 **Figure 2.** Trends in effluent concentrations for (a.) organic carbon (OC), (b.) inorganic carbon,  
 181 and (c.) uranium in sediment columns supplied with different concentrations of OC.

182

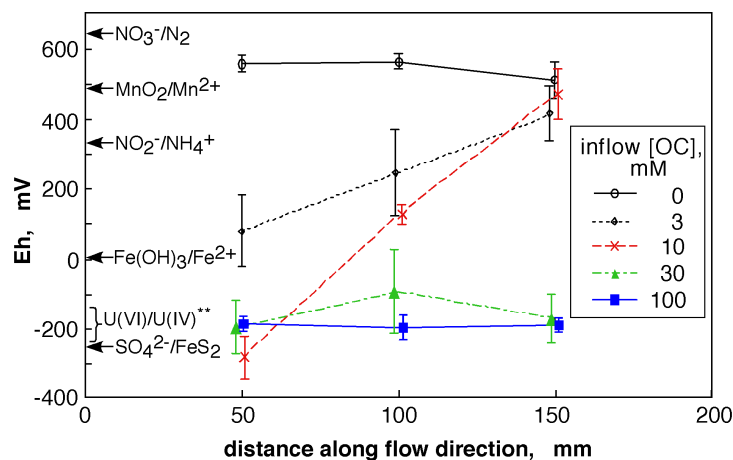
183

184 **Redox electrode measurements.** Stable redox profiles developed by about 2.5 PV of OC  
 185 solution infusion, with trends in Pt electrode data that reflected OC concentrations in influent  
 186 solutions and distances along columns (Figure 3). Calculated Eh (*1, 14*) for nitrate, Mn(IV),  
 187 U(VI), Fe(III), and sulfate reduction are included in Figure 3 for comparison. Although the  
 188 redox measurements are very local and have relatively large uncertainties (1), several conditions

189 are evident. Without addition of OC, redox potentials remained oxidizing, indicating that native  
190 sediment organic matter mineralization rates were low. The 30 and 100 mM OC columns have  
191 redox potentials that have fallen below levels for Mn reduction. The sediment supplied with 3  
192 and 10 mM OC exhibited redox gradients, reflecting OC oxidation during transit.

193

194



195

196

197 **Figure 3.** Redox potential profiles measured with Pt electrodes in columns. Data points are  
198 averages of measurements obtained at 2.5, 5.9, 7.3, 10.0, and 12.7 PV (days 70 to 330) relative to  
199 the start of OC infusion. Range bars indicate standard deviations. Also shown along the Eh axis  
200 are calculated potentials (all at pH 7.3) for nitrate reduction ( $\text{NO}_3^- = 1 \mu\text{M}$ ,  $\text{P}(\text{N}_2) = 0.8 \text{ atm}$ ),  
201 Mn(IV) reduction (pyrolusite,  $\text{Mn}^{2+} = 10 \mu\text{M}$ ), nitrite reduction to ammonia (equimolar N  
202 species), Fe(III) reduction (ferrihydrite,  $\text{Fe}^{2+} = 1 \mu\text{M}$ ), \*\*U(VI) reduction ( $\text{Ca}_2\text{UO}_2(\text{CO}_3)_3 = 1$   
203 mM, U(IV) =  $\text{UO}_2(\text{am})$ , Eh ranges span  $\text{pCO}_2 = 2$  to 1.5 and  $\text{Ca}^{2+} = 0.1$  to 1.0 mM), and sulfate  
204 reduction to pyrite ( $1 \mu\text{M Fe}^{2+}$ ,  $0.1 \text{ mM SO}_4^{2-}$ ).

205

206

207  **$\mu$ -XANES of Mn, Fe, and U.** Time trends in column-averaged characteristic energies of Mn  
208 edges, Fe pre-edges, and U edges are shown in Figure 4, along with reference energies for  
209 oxidation state end members. Sediments with intermediate levels of OC influxes (especially the 3  
210 and 10 mM OC columns) were measured on fewer occasions. The 0, 30, and 100 mM OC

211 treatments exhibited no significant spatial gradients in Mn, Fe, and U oxidation state profiles. In  
212 contrast, the sediment column receiving 10 mM OC solutions showed spatial gradients in  
213 oxidation states (Figure 5). Micro-XANES measurements on the 3 mM OC treated sediment  
214 were only obtained at end of this study (average energies shown in Figure 4), and yielded Mn,  
215 Fe, and U oxidation state profiles (not shown) similar to those of the 10 mM OC treatment.

216 **Manganese oxidation state trends.** Micro-XANES spectra obtained on the initial sediment  
217 showed that Mn occurred in intermediated oxidation states, with very little variability in the K-  
218 edge absorption energies (Figure 4a). Because Mn(III,IV) oxides suppress U bioreduction rates,  
219 and also oxidize uraninite (12), the initial redox status of Mn reflects geochemical conditions  
220 unfavorable for U reduction. Upon starting inflow, highly variable reduction occurred during the  
221 initial stage when only synthetic groundwater (no added OC) was being supplied, reflecting  
222 redox instability often observed upon rewetting sediments (33). This was followed by  
223 systematically OC-dependent trends during the remainder of the experiment (Figure 4a). The  $\mu$ -  
224 XANES spectra obtained on sediments infused with 30 and 100 mM OC had Mn K-edge  
225 energies identical to those of Mn(II) standards (Figure 1a), showing rapid and complete  
226 conversion to Mn(II) by 2.1 PV. Although a relative uncertainty of about 5% is associated with  
227 oxidation states obtained from these Mn  $\mu$ -XANES measurements, corresponding to  $\pm 2$  mmol  
228 Mn kg<sup>-1</sup>, rapid and monotonic reduction of Mn in the 30 and 100 mM OC treatments indicates  
229 that Mn(III,IV) oxides are no longer present, hence are not involved in U reoxidation. Even the  
230 sediment receiving 0 mM OC exhibited gradual Mn reduction, apparently from slow oxidation of  
231 native sediment organic matter. Sediments permeated with 3 and 10 mM OC exhibited  
232 intermediate rates of Mn reduction.

233 Spatial distributions of Mn K-edge energies along the 10 mM OC column at days 174,  
234 266, and 409 are shown in Figure 5a. The presence of a thick wall section prevented collection  
235 of  $\mu$ -XANES data in the vicinity of 100 mm. Note that Mn in the region closest to the OC  
236 supply was more reduced than regions further along the flow path, consistent with redox  
237 electrode data, and reflecting kinetically favorable reduction under OC-limited conditions. Also  
238 note that at the nominally 800  $\mu$ m measurement scale of individual data points, local spatial  
239 variability in Mn oxidations is significant until reduction to Mn(II) is completely achieved.

240 **Iron oxidation state trends.** Based on Fe pre-edge peak centroid energies, initial sediment  
241 samples had a ratio of ferric to total Fe,  $\text{Fe(III)}/\Sigma\text{Fe}$ , of  $0.88 \pm 0.10$ . Even after 400 days (16 PV)  
242 of OC infusion,  $\mu$ -XANES spectra indicated that the  $\text{Fe(III)}/\Sigma\text{Fe}$  remained  $\geq 0.76 \pm 0.10$  (Figure  
243 4b). Thus, in strong contrast to the rapid and complete reduction of Mn in sediments supplied  
244 with the higher levels of OC, overall Fe oxidation states remained relatively unchanged. It  
245 should be noted that although overall Fe oxidation states changed little, some of these changes  
246 were statistically highly significant. In particular, the Fe reduction/oxidation cycles that occurred  
247 from days 112 to 266 for the 30 and 100 mM OC sediments were significant at  $p \leq 0.001$ . The  
248 cause of these Fe redox fluctuations (measured average fluctuations amounted to  $140 \text{ mmol kg}^{-1}$ ,  
249 with a standard deviation of  $60 \text{ mmol kg}^{-1}$ ) was not identified, but possible coupling with U  
250 oxidation state changes is discussed later.

251 The reactive fraction of the sediment Fe is operationally defined, depending on reactions  
252 and time-scales of interest (34, 35). For conditions imposed in this experiment, the citrate-  
253 dithionite extraction appears to provide a fair estimate of the reactive Fe fraction. The citrate-  
254 dithionite extraction removed 21% of this sediment's total Fe (Table S1), and the range of  
255 variation in  $\text{Fe(III)}/\Sigma\text{Fe}$  determined by  $\mu$ -XANES spectroscopy was of similar magnitude

256 (Figure 4b). The resistance to reduction of most of the sediment Fe(III), shown here through  
257 time trends in Fe K-edge  $\mu$ -XANES spectra, is consistent with extraction-based characterizations  
258 of Fe in reducing sediments (36). Lack of detectable spatial trends in overall Fe oxidation state  
259 is shown in the  $\mu$ -XANES profiles from the 10 mM OC column (Figure 5b). All other columns  
260 had similar, Fe(III)-dominated oxidation state profiles. Continued abundance of Fe(III) ( $> 800$   
261  $\text{mmol kg}^{-1}$ ) shows persistence of a high potential oxidizing capacity, with its impact strongly  
262 moderated by low Fe(III) reduction rates. It should also be noted that only a fraction of the total  
263 sediment Fe(III) consisting of poorly crystalline phases is capable of oxidizing U(IV) (14, 16).

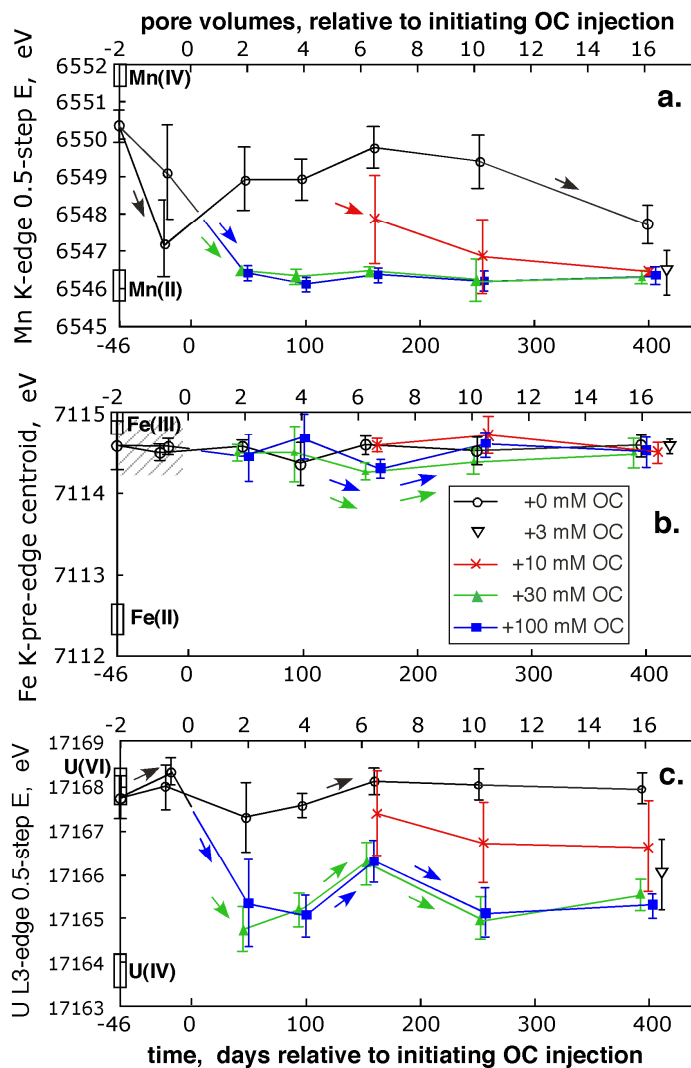
264 **Uranium oxidation state trends.** Uranium  $L_{III}$   $\mu$ -XANES spectra on the original sediment  
265 showed that U occurred largely ( $93 \pm 7\%$ ) as U(VI). The control column supplied with the  
266 synthetic groundwater without OC exhibited only small variations from this initial state  
267 throughout the experiment (Figure 4c). Fluctuations in U  $L_{III}$  edge energies observed at early  
268 stages of the experiment apparently reflect transient redox changes occurring during  
269 establishment of water-saturated conditions. Micro-XANES measurements were obtained at  
270 fewer times for the sediments treated with 3 and 10 mM OC, but their results show levels of U  
271 reduction that are intermediate to those of the 0 versus 30 and 100 mM OC systems. Spatial  
272 profiles of U  $L_{III}$ -edge absorption energies measured in the 10 mM OC-treated sediment (Figure  
273 5c) show U generally occurring in more reduced forms closer to the inlet, remaining more  
274 oxidized toward the outlet end, and retaining a high level of local oxidation state variability. The  
275 heterogeneous nature of local U oxidation states was confirmed through duplicate profile  
276 measurements of U  $L_{III}$ -edge energies on days 266 and 409 (data points with common x values in  
277 Figure 5c). Reproducibility of such duplicate scans demonstrated that X-ray beam induced redox



278 changes were insignificant for not only U, but for Mn and Fe (Figures 5a,b) as well, under our  
279 operating conditions.

280         Rapid U reduction followed by reoxidation was observed in the columns supplied with 30  
281 and 100 mM influent OC concentrations (Figure 4c), similar to U redox changes reported in our  
282 previous study (14). The increases in U oxidation states measured on day 174 (relative to days  
283 62 and 112) in the sediments supplied with 30 and 100 mM OC solutions were significant at  $p \leq$   
284 0.001. Uranium  $\mu$ -XANES spectra obtain on day 266 showed further reduction, also significant  
285 at  $p \leq 0.001$ . The magnitude of U redox fluctuations measured in the 30 and 100 mM OC  
286 systems (days 62 to 266) both amounted to 28% of the full U(IV)/U(VI) edge shift, and are  
287 equivalent to transfers of  $0.60 \text{ mmol e}^- \text{ kg}^{-1}$ . These measured U oxidation state reversals are  
288 complimentary in their directions to the Fe oxidation state variations measured over the same  
289 time interval. Net oxidation of U coincided with net reduction of Fe, and vice versa,  
290 qualitatively supportive of their redox coupling. Because of very similar potentials for  
291 U(VI)/U(IV) and Fe(III)/Fe(II) redox reactions, different Fe minerals have significantly different  
292 impacts on U speciation (14, 16). However, electron transfers of the magnitude measured for U  
293 oxidation state changes are below detection for Fe by  $\mu$ -XANES spectroscopy because of the  
294 high total Fe in the sediment ( $1,185 \text{ mmol kg}^{-1}$ ), and the very large differences in concentrations  
295 of total Fe and U (Fe:U molar ratio = 1,100). Thus, the Fe  $\mu$ -XANES spectroscopy is useful for  
296 identifying the persistence of Fe(III) and its potential availability as a TEA for U reoxidation, but  
297 Fe-U redox coupling could not be stoichiometrically quantified.

298



299

300 **Figure 4.** Time trends of column-average X-ray absorption energies for (a.) Mn K-edge, (b.) Fe

301 K-edge pre-edge peak centroid, and (c.) U L<sub>III</sub>-edge in sediment columns infused with different

302 concentrations of OC. Data points are slightly shifted along the time axis for clarity. Energies of

303 end member oxidation states are shown on the y-axes. Range bars indicate 1 standard deviation.

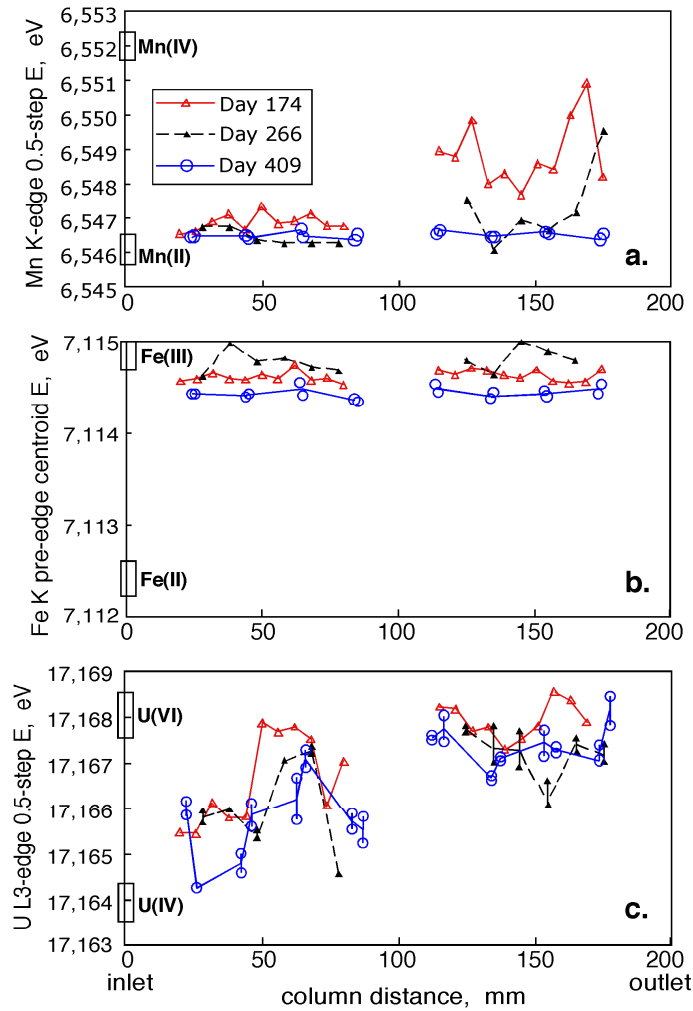
304 The diagonally slashed band in the Fe graph indicates the range of energy shift possible if only

305 the citrate-dithionite extractable Fe is completely reduced. Arrows indicate where energy

306 differences between adjacent times (or relative to the initial sediment in cases where intermediate

307 time measurements were unavailable) are significantly different at  $p \leq 0.001$ .

308



310

311

312 **Figure 5.** Profiles of characteristic X-ray absorption energies for (a.) Mn, (b.) Fe, and (c.) U, in  
 313 the sediment supplied with 10 mM OC, obtained on days 174, 266, and 409 (relative to the start  
 314 of OC injection). Duplicate scans were performed on U profiles obtained on both days 266 and  
 315 409, and shown in data points having common x (distance) values. Reference energies for end  
 316 member oxidation states are indicated along the y-axes. A wall section prevented measurements  
 317 in the center segment of the column.

318

319 Final measurements on day 409 suggest stability of the U redox status in sediments  
320 supplied with 30 and 100 mM OC, but with incomplete reduction. Even during this late phase of  
321 reduction, about 20% of the U remained as U(VI). Factors responsible for incomplete conversion  
322 to U(IV) could include oxidation by Fe(III), and irreversible sorption of some U(VI) on mineral  
323 surfaces (37-39).

324

### 325 **Implications for OC-based, in-situ U reduction**

326 The hypothesis that a reactive Fe(III) fraction served as the TEA for U reoxidation was  
327 supported through these in situ real-time  $\mu$ -XANES measurements of Mn, Fe, and U. Most of the  
328 Fe(III) remained after complete reduction of Mn, and 80% U reduction. In contrast to Mn, the  
329 high mass fraction of Fe(III) in sediments with slow reduction allow Fe(III) phases to persist  
330 under reducing conditions over extended periods of time. Reduction of reactive, poorly  
331 crystalline Fe(III) (hydr)oxides appears necessary before stable U reduction is achievable.  
332 Finally, this work shows that although reoxidized U is later further reduced under a continuous  
333 supply of OC, a U(VI) fraction remained after over 400 days of reduction.

334

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351

### 352 **Supporting Information Available**

353 Elemental analyses of the initial sediment is provided in Table S1. Representative effluent  
354 chemical composition (U, Mn, Fe, and S) data are presented in Table S2. A diagram of the soil  
355 column is presented in Figure S1. This material is available free of charge via the Internet at  
356 <http://pubs.acs.org>.

357

### 358 **Literature Cited**

- 359 (1) Langmuir, D. *Aqueous Environmental Geochemistry*; Prentice-Hall: Upper Saddle River,  
360 NJ, 1997.
- 361 (2) Lovley, D. R.; Roden, E. E.; Phillips, E. J. P.; Woodward, J. C. Enzymatic iron and  
362 uranium reduction by sulfate-reducing bacteria. *Marine Geology* **1993**, *113*, 41-53.
- 363 (3) Anderson, R. T.; Vrionis, H. A.; Ortiz-Bernad, I.; Resch, C. T.; Long, P. E.; Dayvault, R.;  
364 Karp, K.; Marutzky, S.; Metzler, D. R.; Peacock, A.; White, D. C.; Lowe, M.; Lovley, D. R.  
365 Stimulating the in situ activity of *Geobacter* species to remove uranium from the groundwater of  
366 a uranium-contaminated aquifer. *Applied and Environmental Microbiology* **2003**, *69*, 5884-5891.
- 367 (4) Wall, J. D.; Krumholz, L. R. Uranium reduction. *Annual Review of Microbiology* **2006**,  
368 *60*, 149-166.

- 369 (5) Jeon, B. H.; Dempsey, B. A.; Burgos, W. D.; Barnett, M. O.; Roden, E. E. Chemical  
370 reduction of U(VI) by Fe(II) at solid-water interface using natural and synthetic Fe(III) oxides. .  
371 *Environmental Science and Technology* **2005**, *39*, 5642-5649.
- 372 (6) O'Loughlin, E. J.; Kelly, S. D.; Cook, R. E.; Csencsits, R.; Kemner, K. M. Reduction of  
373 uranium(VI) by mixed iron(II)/iron(III) hydroxide (green rust): Formation of UO<sub>2</sub> nanoparticles.  
374 *Environmental Science and Technology* **2003**, *37*, 721-727.
- 375 (7) Beyenal, H.; Sani, R. K.; Peyton, B. M.; Dohnalkova, A. C.; Amonette, J. E.;  
376 Lewandowski, Z. Uranium immobilization by sulfate-reducing biofilms. *Environmental Science*  
377 *and Technology* **2004**, *38*, 2067-2074.
- 378 (8) Abdelouas, A.; Lutze, W.; Nuttall, H. E. Oxidative dissolution of uraninite precipitated  
379 on Navajo sandstone. *Journal of Contaminant Hydrology* **1999**, *36*, 353-375.
- 380 (9) Zhou, P.; Gu, B. Extraction of oxidized and reduced forms of uranium from contaminated  
381 soils: Effects of carbonate concentration and pH. *Environmental Science and Technology* **2005**,  
382 *39*, 4435-4440.
- 383 (10) Senko, J. M.; Istok, J. D.; Suflita, J. M.; Krumholz, L. R. In-situ evidence for uranium  
384 immobilization and remobilization. *Environmental Science and Technology* **2002**, *36*, 1491-  
385 1496.
- 386 (11) Istok, J. D.; Senko, J. M.; Krumholz, L. R.; Watson, D.; Bogle, M. A.; Peacock, A.;  
387 Chang, Y.-J.; White, D. C. In situ bioreduction of technitium and uranium in a nitrate-  
388 contaminated aquifer. *Environmental Science and Technology* **2004**, *38*, 468-475.
- 389 (12) Fredrickson, J. K.; Zachara, J. M.; Kennedy, D. W.; Liu, C.; Duff, M. C.; Hunter, D. B.;  
390 Dohnalkova, A. Influence of Mn oxides on the reduction of uranium (VI) by the metal-reducing  
391 bacterium *Shewanella putrefaciens*. . *Geochimica Cosmochimica Acta* **2002**, *66*, 3247-3262.
- 392 (13) Sani, R. K.; Peyton, B. M.; Dohnalkova, A.; Amonette, J. E. Reoxidation of reduced  
393 uranium with iron(III) (hydr)oxides under sulfate-reducing conditions. *Environmental Science*  
394 *and Technology* **2005**, *39*, 2059-2066.
- 395 (14) Wan, J.; Tokunaga, T. K.; Brodie, E. L.; Wang, Z.; Zheng, Z.; Herman, D. J.; Hazen, T.  
396 C.; Firestone, M. K.; Sutton, S. R. Reoxidation of bioreduced uranium under reducing  
397 conditions. *Environmental Science and Technology* **2005**, *39*, 6162-6169.
- 398 (15) Senko, J. M.; Mohamed, Y.; Dewers, T. A.; Krumholz, L. R. Role of Fe(III) minerals in  
399 nitrate-dependent microbial U(IV) oxidation. *Environmental Science and Technology* **2005**, *39*,  
400 2529-2536.
- 401 (16) Ginder-Vogel, M.; Criddle, C. S.; Fendorf, S. Thermodynamic constraints on the  
402 oxidation of biogenic UO<sub>2</sub> by Fe(III) (hydr)oxides. *Environmental Science and Technology*  
403 **2006**, *40*, 3544-3550.
- 404 (17) Brodie, E. L.; DeSantis, T. Z.; Joyner, D. C.; Baek, S. M.; Larsen, J. T.; Anderson, G. L.;  
405 Hazen, T. C.; Richardson, P. M.; Herman, D. J.; Tokunaga, T. K.; Wan, J. M.; Firestone, M. K.  
406 Application of a high-density oligonucleotide microarray approach to study bacterial population  
407 dynamics during uranium reduction and reoxidation. *Applied and Environmental Microbiology*  
408 **2006**, *72*, 6288-6298.
- 409 (18) Roden, E. E.; Zachara, J. M. Microbial reduction of crystalline iron(III) oxides: Influence  
410 of oxide surface area and potential for cell growth. *Environmental Science and Technology* **1996**,  
411 *30*, 1618-1628.
- 412 (19) Lovley, D. R.; Phillips, E. J. P. Rapid assay for microbially reducible ferric iron in  
413 aquatic sediments. *Applied and Environmental Microbiology* **1987**, *53*, 1536-1540.
- 414 (20) Zavarin, M. In *Soil Science*; University of California: Berkeley, 1999; Vol. Ph. D., p 279.

- 415 (21) Bertsch, P. M.; Hunter, D. B. Applications of synchrotron-based x-ray microprobes.  
416 *Chemical Reviews* **2001**, *101*, 1809-1842.
- 417 (22) Sutton, S. R.; Bertsch, P. M.; Newville, M.; Rivers, M.; Lanzirrotti, A.; Eng, P.  
418 Microfluorescence and microtomography analyses of heterogeneous earth and environmental  
419 materials. In *Applications of Synchrotron Radiation in Low-Temperature Geochemistry and*  
420 *Environmental Science*; Fenter, P. A., Rivers, M. L., Sturchio, N. C., Sutton, S. R., Eds.;  
421 Mineralogical Society of America: Chantilly, VA, 2002; Vol. 49, pp 429-483.
- 422 (23) Ross, D. S.; Hales, H. C.; Shea-McCarthy, G. C.; Lanzirrotti, A. Sensitivity of soil  
423 manganese oxides: XANES spectroscopy may cause reduction. *Soil Science Society of America*  
424 *Journal* **2001**, *65*, 744-752.
- 425 (24) Riggs-Gelasco, P. J.; Mei, R.; Ghanotakis, D. F.; Yocum, C. F.; Penner-Hahn, J. E. X-ray  
426 absorption spectroscopy of calcium-substituted derivatives of the oxygen-evolving complex of  
427 Photosystem II. *Journal of the American Chemical Society* **1996**, *118*, 2400-2410.
- 428 (25) Gorry, P. A. General least-squares smoothing and differentiation by the convolution  
429 (Savitzky-Golay) method. *Analytical Chemistry* **1990**, *62*, 570-573.
- 430 (26) Negra, C.; Ross, D. S.; Lanzirrotti, A. Oxidation behavior of soil manganese: Interactions  
431 among abundance, oxidation state, and pH. *Soil Science Society of America Journal* **2005**, *69*,  
432 87-95.
- 433 (27) Bajt, S.; Sutton, S. R.; Delaney, J. S. X-ray microprobe analysis of iron oxidation states  
434 in silicates and oxides using X-ray absorption near edge structure (XANES). *Geochimica*  
435 *Cosmochimica Acta* **1994**, *58*, 5209-5214.
- 436 (28) Wilke, M.; Farges, F.; Petit, P.-E.; Brown, G. E., Jr.; Martin, F. Oxidation state and  
437 coordination of Fe in minerals: An Fe K-XANES spectroscopic study. *American Mineralogist*  
438 **2001**, *86*, 716-730.
- 439 (29) Berry, A. J.; O'Neill, H. S. C.; Jayasuriya, K.; Campbell, S. J.; Foran, G. J. XANES  
440 calibrations for the oxidation state of iron in silicate glass. *American Mineralogist* **2003**, *88*, 967-  
441 977.
- 442 (30) La Force, M. J.; Fendorf, S. Solid-phase iron characterization during common selective  
443 sequential extractions. *Soil Science Society of America Journal* **2000**, *64*, 1608-1615.
- 444 (31) Duff, M. C.; Morris, D. E.; Hunter, D. B.; Bertsch, P. M. Spectroscopic characterization  
445 of uranium in evaporation basin sediments. *Geochimica Cosmochimica Acta* **2000**, *64*, 1535-  
446 1550.
- 447 (32) Finneran, K. T.; Housewright, M. E.; Lovley, D. R. Multiple influences of nitrate on  
448 uranium solubility during bioremediation of uranium-contaminated subsurface sediments.  
449 *Environmental Microbiology* **2002**, *4*, 510-516.
- 450 (33) Bartlett, R.; James, B. Studying dried, stored soil samples -some pitfalls. *Soil Science*  
451 *Society of America Journal* **1980**, *44*, 721-724.
- 452 (34) Roden, E. E. Analysis of long-term bacterial vs. chemical Fe(III) oxide reduction  
453 kinetics. *Geochimica Cosmochimica Acta* **2004**, *68*, 3205-3216.
- 454 (35) Hyacinthe, C.; Bonneville, S.; Van Cappellen, P. Reactive iron(III) in sediments:  
455 Chemical versus microbial extractions. *Geochimica Cosmochimica Acta* **2006**, *70*, 4166-4180.
- 456 (36) Lovley, D. R.; Phillips, E. J. P. Availability of ferric iron for microbial reduction in  
457 bottom sediments of the freshwater tidal Potomac River. *Applied and Environmental*  
458 *Microbiology* **1986**, *52*, 751-757.

- 459 (37) Jeon, B. H.; Kelly, S. D.; Kemner, K. M.; Barnett, M. O.; Burgos, W. D.; Dempsey, B.  
460 A.; Roden, E. E. Microbial reduction of U(VI) at the solid-water interface. *Environmental*  
461 *Science and Technology* **2004**, *38*, 5649-5655.
- 462 (38) Giammar, D. E.; Hering, J. G. Time scales for sorption-desorption and surface  
463 precipitation of uranyl on goethite. *Environmental Science and Technology* **2001**, *35*, 3332-3337.
- 464 (39) Ortiz-Bernad, I.; Anderson, R. T.; Vrionis, H. A.; Lovley, D. R. Resistance of solid-phase  
465 U(VI) to microbial reduction during in situ bioremediation of uranium-contaminated  
466 groundwater. *Environmental Microbiology* **2004**, *70*, 7558-7560.
- 467  
468  
469