In situ Bioreduction of Uranium (VI) to Submicromolar

Levels and Reoxidation by Dissolved Oxygen

Supporting Information

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Bacterial Community Analysis

A) Groundwater samples.

Total bacterial genomic DNA was extracted from the filters as described previously (Hwang et al.,

2006). The SSU rRNA genes were amplified in a PTC-200 Peltier Thermal Cycler (MJ Research) with

the primer pair FD1 F (5' AGA GTT TGA TCC TGG CTC AG 3') and 1540R (5' AAG GAG GTG

ATC CAG CC 3'). The PCR reaction (25 ul) contained 12.5 µL BioMix (Bioline, Randolph, MA), 1µL

each primer, 9.5 µL sterilized Milli-Q water, 1µL purified DNA (5-10 ng). To minimize PCR-induced

artifacts, the optimal number of cycles was determined and five PCR reactions were combined prior to

cloning as described previously (Qui et al. 2001). The PCR parameters were as follows: 80˚C for 1.5

min; 94˚C for 2 min; 94˚C for 30s; 58˚C for 1 min; 72˚C for 1 min; 20 cycles; 72˚C for 7 min. An

aliquot of 5 µL of PCR product was run in a 0.8% agarose TAE gel stained with ethidium bromide to

evaluate the quality of the amplified fragment.

PCR products of the SSU rRNA genes were purified using a Promega Kit (Promega, Madison,

WI). The purified fragments were cloned using the vector PCR 2.1-TOPO and *Escherichia coli*

DH5_\plain[TM]-T1[R] competent cells according to the manufacturer's instructions (Invitrogen, Carlsbad,

CA). From each of the libraries approximately 100 white colonies were randomly selected, and the

cloned inserts were amplified with vector-specific primers M13 forward and M13 reverse (30 cycles of

the PCR parameters described above, but with an annealing temperature of 60˚C and a final extension

time of 10 minutes). The resulting amplification products were analyzed as described above. PCR

products (20µl) amplified with vector-specific primers were purified with a Montage PCR_{u96} plate

according to the manufacturer's instructions (Millipore, Bedford, MA). DNA sequences were

determined with a DYEnamic ET Terminator Cycle Sequencing Kit (Amersham Biosciences,

Piscataway, NJ) according to manufacturer's instructions at 1:4 dilutions with an internal primer (529r).

Sequence reaction products were analyzed on the ABI model 3100 and 3730 DNA sequencer (Applied

Biosystems). DNA sequences were assembled and edited using the Sequencher[TM] program (v. 4.0,

Gene Codes Corporation, Ann Arbor, MI). The SSU rDNA sequence identification was performed with

the BLASTn server (http://www.ncbi.nlm.nih.gov/BLAST) and the RDPII

(http://rdp.cme.msu.edu/index.jsp). Unique SSU rDNA clones from each library were identified and

designated as OTUs (operational taxonomic unit) at a 97% sequence identity level. The sequences were

aligned with Clustal W (Thompson et al. 1994). Phylogenetic trees were constructed with distance

matrices and the neighbor-joining method, and bootstrap analysis was performed with programs within

53 MEGA version 2.1 (Kumar et al. 2001).

54 ***B) Sediment Samples***

55 Sediments were collected from the wells in the treatment zone by a well surging operation, as described

56 previously (Wu et al., 2006b). Total bacterial DNA was extracted from 0.5 g of sediments with the Fast

57 DNA spin kit for soil (BIO 101, Carlsbad, CA) following the manufacturer's instructions.

58   16S rRNA genes were amplified using the universal primers 27F (5'-

59 AGAGTTTGATCMTGGCTCAF-3') and 1392R (5'-ACGGGCGGTGTGTRC-3'). Thermocycler

60 conditions were as follows: 95ºC for 5 min; 95 ºC for 1 min, 59 ºC for 1 min, 72ºC for 1:40 min, for 28

61 cycles; 72 ºC for 10 min. PCR products were analyzed in a 1.5% agarose TAE gel and the size of the

62 product was confirmed. To control for PCR bias, four replicate PCR reactions were prepared for each

63 sample and then compiled. The combined PCR products (200 µl) were purified using a Qiaquick PCR

64 purification kit (Qiagen Inc., Valencia, CA, USA). The concentrated products were then run in a 1%

65 agarose gel, excised, and extracted with a Qiaquick gel extraction kit (Qiagen Inc., Valencia, CA).

66 Adenine overhangs were added to the PCR products by incubating a mixture of 18.8mM Tris HCl pH

67 8.4, 47mM KCl, 0.93mM dATP and 0.5 U of taq polymerase for10 min at 72 ºC. Prepared PCR

68 products were then cloned using the Topo TA Clonig kit for sequencing following manufacturer's

69 instructions (Invitrogen Life Technologies, Carlsbad, CA, USA). Single read sequencing was conducted

70 by Macrogen Inc (Seoul, Korea) using the internal primer 27F.

71   The raw sequence data (Ab1 files) were submitted to the Pipeline application of the Ribosomal

72 Database project (RDP at www.rdp.cme.msu.edu) (Cole et al., 2005, 2007). Bases were called using

73 PHRED (Ewing et al., 1998) and vector sequence removed with LUCY (Chou et al., 2001). The

74 resulting sequences were aligned to the RDP model and rRNA distances matrices were generated and

75 corrected using the Jukes – Cantor correction. Sequences were also downloaded with a Q20 quality

76 mask to be later classified using the RDP classifier. The sequences were classified with the classifier

77 application of the RDP using an 80% confidence value.

78 For methods section:

79 **Functional Gene Microarray**

80 A 50mer functional gene microarray containing 25,098 genes involved in biogeochemical cycling of C,

81 N, S, organic contaminant degradation, and metal homeostasis (Rhee et al., 2004; Li et al., 2005;

82 Liebich et al. 2006) was used to examine microbial communities from ground water. Samples were

83 obtained as described above. DNA was extracted from filters by the method of Zhou et al. (1996) and

84   50 ng aliquots of DNA were amplified, labeled and hybridized as described previously (Wu et al.,

85   2006).

Most Probable Number (MPN) method

87   Denitrifying bacteria, FeRB and SRB were enumerated using the Most Probable Number (MPN)

88   technique with five tubes for each dilution. Anaerobic pressure tubes (27 mL) containing 10 ml basal

89   medium were sealed with butyl rubber stoppers with aluminum caps. The basal medium contained the

90   following components (per liter): NH_4Cl, 0.5g; NaCl, 0.4g; $NaHCO_3$, 0.55g; and mineral solution, 100

91   ml.   The mineral solution contained (per liter): $MnCl_2$, 0.4 g; $MgSO_4$, 1.5 g; $CaCl_2$, 0.5 g; and yeast

92   extract, 0.02g. The medium was prepared under a N_2-CO_2 (99:1, vol/vol) atmosphere and distributed to

93   each pressure tube (10 mL per tube). After autoclaving, a sterile trace element solution (0.4 ml) and a

94   sodium trimetaphosphate solution (50 mM, 0.025 ml) was added into each tube to obtain pH 7.0. The

95   trace element solution contained (per liter): HCl (12N), 6.4 mL; $FeCl_2 \cdot 4H_2O$, 0.3g; $ZnSO_4 \cdot H_2O$, 0.1g;

96    $MnSO_4$, 0.085g; HB, 0.06g; $CoCl_2 \cdot 6H_2O$, 0.02g; $CuSO_4$, 0.004g; $NiSO_4 \cdot 6H_2O$, 0.028 g; and

97    $NaMoO_3 \cdot 2H_2O$, 0.04g. The electron acceptor solution was supplemented to the tubes to obtain 5 mM

98   Fe-citrate for FeRB, 1.0 g/L of sodium thioglycollate and 5 g/l of $FeSO_4$ for SRB, and 1.0 g/L of $NaNO_3$

99   for denitrifiers, respectively.   Ethanol solution (1 M) was added to each tube to give a final

100   concentration of 10 mM. Groundwater was pumped from the wells into anaerobic pressure tubes pre-

101   filled with nitrogen gas. The sample was then serially diluted in MPN tubes. The inoculated tubes were

102   incubated at ambient temperature for two months. Tubes were compared to controls for scoring as

103   positive or negative for production of gas in denitrifying tubes, color change in FeRB tubes, and

104   production of black Fe(II) precipitates in SRB tubes. FeRB and SRB tubes were also analyzed and

105   scored for methane production. Methane production in MPN tubes for FeRB and SRB was considered

106   indicative of the presence of methanogens.

107

XANES Measurements at MR-CAT

109   Some of the XANES analysis were carried out at Argonne National Laboratory (ANL) and

110   others at Stanford University. Samples measured at ANL are shown in Supplemental Figure 1. Sediment

111   samples from the wells were collected in anaerobic serum bottles with He or Ar headspace and stored at

112   4 $^{\circ}$C in a refrigerator. They were centrifuged to separate the sediments from the supernatant within a

113   Coy(R) Anoxic Chamber filled with N_2 and H_2 mixture, mounted under anoxic conditions into

114   plexiglass sample holders with x-ray transparent windows of Kapton film covered with Kapton tape,

115   and stored at 4° C in canning jars filled with anoxic atmosphere for at most 3 days until measurements

116   were made at the MR-CAT (Segre et al., 2000) beamline at the Advance Photon Source, Argonne

117   National Laboratory, USA. The sealed samples were exposed to oxygen only briefly to place them in

118   the sample chamber at the beamline which was purged with N_2 gas. When exposed to atmospheric

119   conditions, sealed samples have been shown to preserve the anoxic integrity of the samples for more

120   than 24 hours. The average valence states of U within the moist sediments were measured under anoxic

121   conditions with no pretreatment of the sediments.

122   The MR-CAT insertion device beamline parameters were as follows. The insertion device was

123   operated on the third harmonic with a taper of approximately 2 keV to produce an incident x-ray

124   intensity as uniform as possible over the scanned x-ray region from 17,000 eV to 18,000 eV. A double

125   crystal Si(111) monochromator was used to select the x-ray energy. A Rh harmonic x-ray rejection

126   mirror was used to eliminate x-rays with higher harmonic energies. The x-ray profile at the sample was

127   approximately 0.7 mm square. The incident, transmitted, and fluorescence x-ray intensities were

128   measured with pure N_2, mixture of 90% N_2 and 10% Ar, and pure Ar filled ionization chambers,

129   respectively. The fluorescence ionization chamber was used with solar slits and an Al filter in the Stern-

130   Heald geometry (Stern et al, 1979; Kemner et al., 1994) performed with 50% attenuation in x-ray

131   intensity on reference samples indicated that the nonlinearity in the detectors was less than 0.5%. A

132   energy reference spectrum from a hydrogen uranyl phosphate standard was measured with each energy

133   scan. This reference was measured after the sample using a reference ionization chamber filled with a

134   mixture of 90% Ar and 10% N_2.

135   The monochromator was scanned continuously to collect quick spectra within ~ 2 minutes.

136   Long x-ray exposure times to the moist samples showed some changes in the XANES spectra.

137   Therefore two scans, resulting in less than 5% change in the spectra were collected from many different

138   regions of the uniform sample consisting of fine particulates surged from the wells. The spectra from

139   each sample were averaged to increase the signal to noise ratio within the final spectra. The averaged

140   spectra are shown in Supplemental Figure 1.

141   The XANES spectra were modeled with a linear combination of standard spectra with the

142   software Athena (Ravel et al., 2005) which uses IFEFFIT methods (Newville, et al., 2001). Two

143   different U(VI) and U(IV) spectra were used as possible standards as needed to reproduce the measured

144   spectra. The U(VI) standards are aqueous uranyl nitrate at low pH (denoted U(VI) Std-1) and a

145 hydrogen uranyl phosphate mineral (denoted U(VI) Std-2). The U(IV) standards are a natural uraninite

146 mineral (denoted U(IV) Std-1) and abiotically produced uraninite particles (denoted U(IV) Std-2)

147 (O'Loughlin, et al., 2000). These standards are shown in Supplemental Figure 1.

148
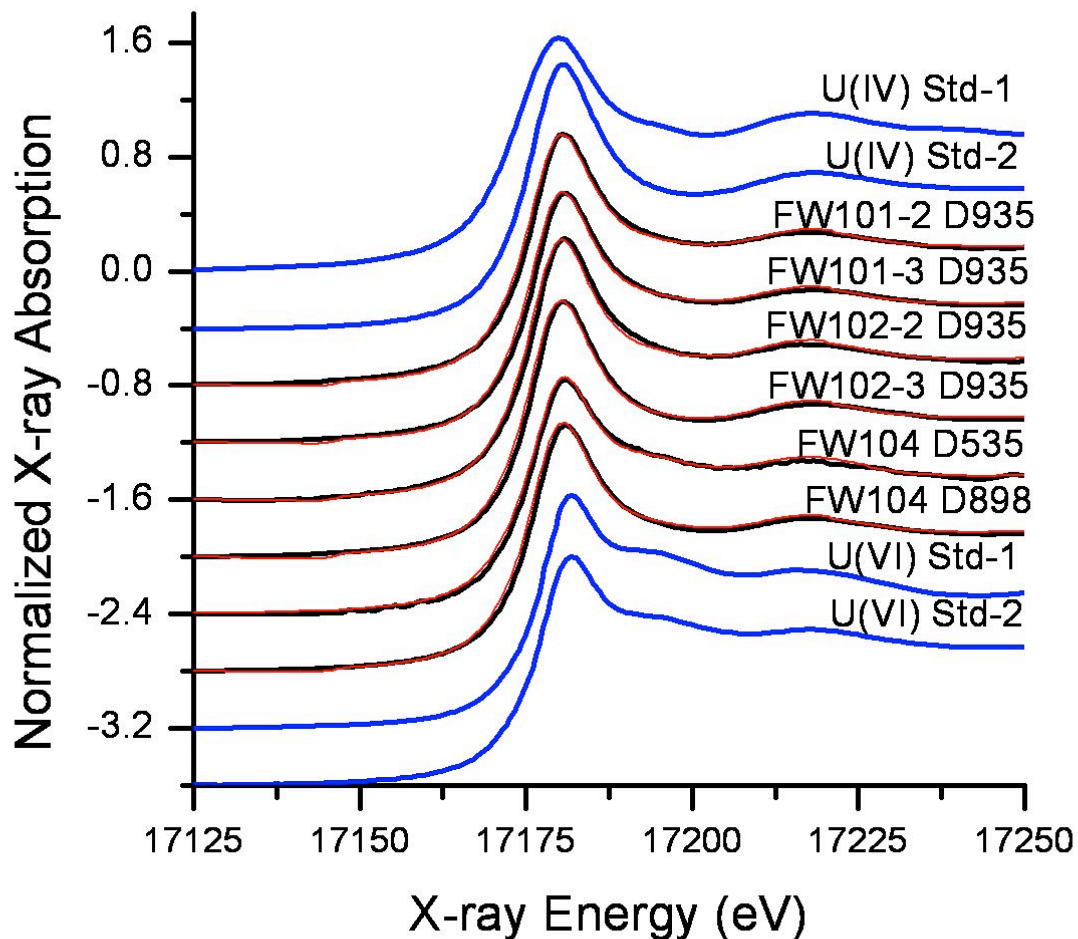
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150   Supplemental Figure 1.XANES analysis of selected samples (black) and linear

151   combination fitting (red) with U(IV) and U(VI) standards (blue).

152

153   The linear combinations are shown with the measured spectra in Supplemental Figure 1. The

154 average U(IV) percentage is listed in Table 1 with the remaining percentage as U(VI). As shown in

155 Supplemental Figure 1, the XANES spectra depend largely on the U valence state but also to a lesser

156 extent on the U speciation. The reference spectra used in the linear combination fitting are from species

157 that are surely somewhat different from the actual species within the sediment samples, causing some

158 systematic error in the percentage of U(IV) to U(VI) within the sediment sample. Based on the

159 differences of the XANES spectra for different U(VI) and U(IV) species, we generously estimate the

160 accuracy of this method to be 10%.

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