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), 1 µL each primer, 9.5 µL sterilized Milli-Q water, 1 µL 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\textsuperscript{TM}-T1\textsuperscript{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 (20 µl) amplified with vector-specific primers were purified with a Montage PCR\textsuperscript{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\textsuperscript{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
MEGA version 2.1 (Kumar et al. 2001).

**B) Sediment Samples**

Sediments were collected from the wells in the treatment zone by a well surging operation, as described previously (Wu et al., 2006b). Total bacterial DNA was extracted from 0.5 g of sediments with the Fast DNA spin kit for soil (BIO 101, Carlsbad, CA) following the manufacturer’s instructions.

16S rRNA genes were amplified using the universal primers 27F (5’- AGAGTTTGATCMTGGCTCAF-3’) and 1392R (5’-ACGGGCGGTGTGTRC-3’). Thermocycler conditions were as follows: 95 ºC for 5 min; 95 ºC for 1 min, 59 ºC for 1 min, 72 ºC for 1:40 min, for 28 cycles; 72 ºC for 10 min. PCR products were analyzed in a 1.5% agarose TAE gel and the size of the product was confirmed. To control for PCR bias, four replicate PCR reactions were prepared for each sample and then compiled. The combined PCR products (200 µl) were purified using a Qiaquick PCR purification kit (Qiagen Inc., Valencia, CA, USA). The concentrated products were then run in a 1% agarose gel, excised, and extracted with a Qiaquick gel extraction kit (Qiagen Inc., Valencia, CA).

Adenine overhangs were added to the PCR products by incubating a mixture of 18.8mM Tris HCl pH 8.4, 47mM KCl, 0.93mM dATP and 0.5 U of taq polymerase for 10 min at 72 ºC. Prepared PCR products were then cloned using the Topo TA Clonig kit for sequencing following manufacturer’s instructions (Invitrogen Life Technologies, Carlsbad, CA, USA). Single read sequencing was conducted by Macrogen Inc (Seoul, Korea) using the internal primer 27F.

The raw sequence data (Ab1 files) were submitted to the Pipeline application of the Ribosomal Database project (RDP at www.rdp.cme.msu.edu) (Cole et al., 2005, 2007). Bases were called using PHRED (Ewing et al., 1998) and vector sequence removed with LUCY (Chou et al., 2001). The resulting sequences were aligned to the RDP model and rRNA distances matrices were generated and corrected using the Jukes – Cantor correction. Sequences were also downloaded with a Q20 quality mask to be later classified using the RDP classifier. The sequences were classified with the classifier application of the RDP using an 80% confidence value.

For methods section:

**Functional Gene Microarray**

A 50mer functional gene microarray containing 25,098 genes involved in biogeochemical cycling of C, N, S, organic contaminant degradation, and metal homeostasis (Rhee et al., 2004; Li et al., 2005; Liebich et al. 2006) was used to examine microbial communities from ground water. Samples were obtained as described above. DNA was extracted from filters by the method of Zhou et al. (1996) and
50 ng aliquots of DNA were amplified, labeled and hybridized as described previously (Wu et al., 2006).

**Most Probable Number (MPN) method**

Denitrifying bacteria, FeRB and SRB were enumerated using the Most Probable Number (MPN) technique with five tubes for each dilution. Anaerobic pressure tubes (27 mL) containing 10 ml basal medium were sealed with butyl rubber stoppers with aluminum caps. The basal medium contained the following components (per liter): NH₄Cl, 0.5g; NaCl, 0.4g; NaHCO₃, 0.55g; and mineral solution, 100 ml. The mineral solution contained (per liter): MnCl₂, 0.4 g; MgSO₄, 1.5 g; CaCl₂, 0.5 g; and yeast extract, 0.02g. The medium was prepared under a N₂-CO₂ (99:1, vol/vol) atmosphere and distributed to each pressure tube (10 mL per tube). After autoclaving, a sterile trace element solution (0.4 ml) and a sodium trimetaphosphate solution (50 mM, 0.025 ml) was added into each tube to obtain pH 7.0. The trace element solution contained (per liter): HCl (12N), 6.4 mL; FeCl₂·4H₂O, 0.3g; ZnSO₄·H₂O, 0.1g; MnSO₄, 0.085g; HB, 0.06g; CoCl₂·6H₂O, 0.02g; CuSO₄, 0.004g; NiSO₄·6H₂O, 0.028 g; and NaMoO₃·2H₂O, 0.04g. The electron acceptor solution was supplemented to the tubes to obtain 5 mM Fe-citrate for FeRB, 1.0 g/L of sodium thioglycollate and 5 g/l of FeSO₄ for SRB, and 1.0 g/L of NaNO₃ for denitrifiers, respectively. Ethanol solution (1 M) was added to each tube to give a final concentration of 10 mM. Groundwater was pumped from the wells into anaerobic pressure tubes prefilled with nitrogen gas. The sample was then serially diluted in MPN tubes. The inoculated tubes were incubated at ambient temperature for two months. Tubes were compared to controls for scoring as positive or negative for production of gas in denitrifying tubes, color change in FeRB tubes, and production of black Fe(II) precipitates in SRB tubes. FeRB and SRB tubes were also analyzed and scored for methane production. Methane production in MPN tubes for FeRB and SRB was considered indicative of the presence of methanogens.

**XANES Measurements at MR-CAT**

Some of the XANES analysis were carried out at Argonne National Laboratory (ANL) and others at Stanford University. Samples measured at ANL are shown in Supplemental Figure 1. Sediment samples from the wells were collected in anaerobic serum bottles with He or Ar headspace and stored at 4 °C in a refrigerator. They were centrifuged to separate the sediments from the supernatant within a Coy(R) Anoxic Chamber filled with N₂ and H₂ mixture, mounted under anoxic conditions into plexiglass sample holders with x-ray transparent windows of Kapton film covered with Kapton tape, and stored at 4° C in canning jars filled with anoxic atmosphere for at most 3 days until measurements.
were made at the MR-CAT (Segre et al., 2000) beamline at the Advance Photon Source, Argonne National Laboratory, USA. The sealed samples were exposed to oxygen only briefly to place them in the sample chamber at the beamline which was purged with N\textsubscript{2} gas. When exposed to atmospheric conditions, sealed samples have been shown to preserve the anoxic integrity of the samples for more than 24 hours. The average valence states of U within the moist sediments were measured under anoxic conditions with no pretreatment of the sediments.

The MR-CAT insertion device beamline parameters were as follows. The insertion device was operated on the third harmonic with a taper of approximately 2 keV to produce an incident x-ray intensity as uniform as possible over the scanned x-ray region from 17,000 eV to 18,000 eV. A double crystal Si(111) monochromator was used to select the x-ray energy. A Rh harmonic x-ray rejection mirror was used to eliminate x-rays with higher harmonic energies. The x-ray profile at the sample was approximately 0.7 mm square. The incident, transmitted, and fluorescence x-ray intensities were measured with pure N\textsubscript{2}, mixture of 90% N\textsubscript{2} and 10% Ar, and pure Ar filled ionization chambers, respectively. The fluorescence ionization chamber was used with solar slits and an Al filter in the Stern-Heald geometry (Stern et al, 1979; Kemner et al., 1994) performed with 50% attenuation in x-ray intensity on reference samples indicated that the nonlinearity in the detectors was less than 0.5%. A energy reference spectrum from a hydrogen uranyl phosphate standard was measured with each energy scan. This reference was measured after the sample using a reference ionization chamber filled with a mixture of 90% Ar and 10% N\textsubscript{2}.

The monochromator was scanned continuously to collect quick spectra within ~ 2 minutes. Long x-ray exposure times to the moist samples showed some changes in the XANES spectra. Therefore two scans, resulting in less than 5% change in the spectra were collected from many different regions of the uniform sample consisting of fine particulates surged from the wells. The spectra from each sample were averaged to increase the signal to noise ratio within the final spectra. The averaged spectra are shown in Supplemental Figure 1.

The XANES spectra were modeled with a linear combination of standard spectra with the software Athena (Ravel et al., 2005) which uses IFEFFIT methods (Newville, et al., 2001). Two different U(VI) and U(IV) spectra were used as possible standards as needed to reproduce the measured spectra. The U(VI) standards are aqueous uranyl nitrate at low pH (denoted U(VI) Std-1) and a
hydrogen uranyl phosphate mineral (denoted U(VI) Std-2). The U(IV) standards are a natural uraninite mineral (denoted U(IV) Std-1) and abiotically produced uraninite particles (denoted U(IV) Std-2) (O’Loughlin, et al., 2000). These standards are shown in Supplemental Figure 1.

Supplemental Figure 1. XANES analysis of selected samples (black) and linear combination fitting (red) with U(IV) and U(VI) standards (blue).

The linear combinations are shown with the measured spectra in Supplemental Figure 1. The average U(IV) percentage is listed in Table 1 with the remaining percentage as U(VI). As shown in Supplemental Figure 1, the XANES spectra depend largely on the U valence state but also to a lesser extent on the U speciation. The reference spectra used in the linear combination fitting are from species that are surely somewhat different from the actual species within the sediment samples, causing some systematic error in the percentage of U(IV) to U(VI) within the sediment sample. Based on the
differences of the XANES spectra for different U(VI) and U(IV) species, we generously estimate the
accuracy of this method to be 10%.

References

Ewing, B.; Green, P. Base-calling of automated sequencer traces using phred. II. Error probabilities.


