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Sorption and bioreduction of hexavalent uranium at a military facility by the Chesapeake Bay

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At the Aberdeen Proving Ground in Maryland, USA, migration of depleted uranium into the Chesapeake Bay is limited by rapid sorption of the radionuclide to natural organic matter followed by slow biological reduction of water-soluble U(VI) to the insoluble and less toxic U(IV) species.

Abstract

Directly adjacent to the Chesapeake Bay lies the Aberdeen Proving Ground, a U.S. Army facility where testing of armor-piercing ammunitions has resulted in the deposition of >70,000 kg of depleted uranium (DU) to local soils and sediments. Results of previous environmental monitoring suggested limited mobilization in the impact area and no transport of DU into the nation's largest estuary. To determine if physical and biological reactions constitute mechanisms involved in limiting contaminant transport, the sorption and biotransformation behavior of the radionuclide was studied using geochemical modeling and laboratory microcosms (500 ppb U(VI) initially). An immediate decline in dissolved U(VI) concentrations was observed under both sterile and non-sterile conditions due to rapid association of U(VI) with natural organic matter in the sediment. Reduction of U(VI) to U(IV) occurred only in non-sterile microcosms. In the non-sterile samples, intrinsic bioreduction of uranium involved bacteria of the order *Clostridiales* and was only moderately enhanced by the addition of acetate (41% vs. 56% in 121 days). Overall, this study demonstrates that the migration of depleted uranium from the APG site into the Chesapeake Bay may be limited by a combination of processes that include rapid sorption of U(VI) species to natural organic matter, followed by slow, intrinsic bioreduction to U(IV).

Keywords: Uranium; Sorption; Bioreduction; Natural organic matter; Humic substances

1. Introduction

The Chesapeake Bay is the largest estuary in the U.S. and represents an expansive and unique ecosystem. The Aberdeen Proving Ground (APG) in Maryland located directly on the bay (Fig. 1), is a military facility, where testing of armor-piercing ammunitions has resulted in the deposition of >70,000 kg of depleted uranium (DU) to surface soils and sediments (Oxenberg, 1997). Because DU is a heavy metal toxic to biota and has a radioactive half-life of 4.5 billion years, it is desirable to control or remove pollution sources from the site. However, localization and removal of DU projectiles historically has been

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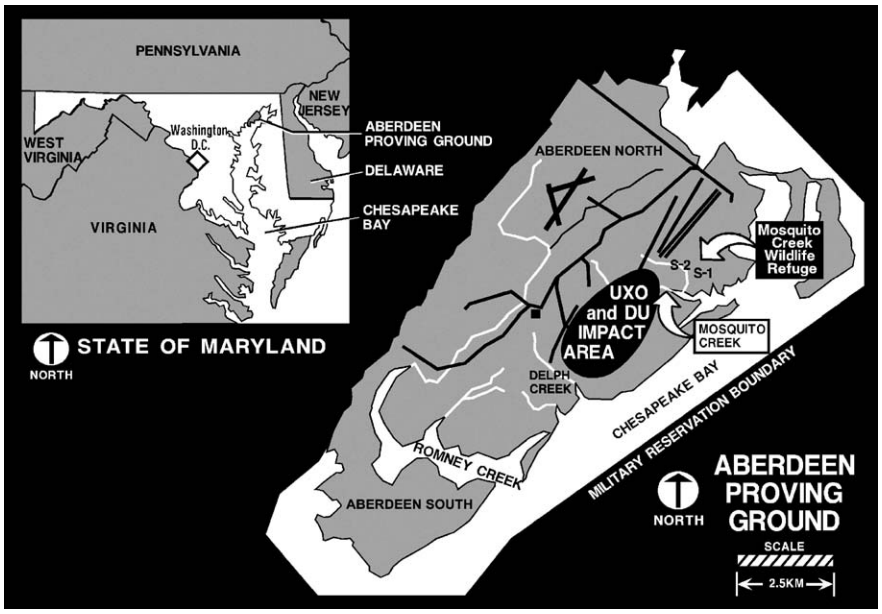

Fig. 1. Geographic map showing the location of the Aberdeen Proving Ground relative to the Chesapeake Bay (insert at the top, left) and the sampling locations S1 and S2 in the Mosquito Creek Wildlife Refuge near the unexploded ordnances (UXO) and depleted uranium (DU) impact area.

challenging due to the presence of unexploded ordnances (UXO) that pose a serious threat to local site workers. The majority of the DU inventory at APG is distributed near the surface and at shallow depths in soils and sediments of an area between Mosquito Creek and Delph Creek. This impact area features diverse ecosystems including terrestrial, fresh water, brackish water, and marine aquatic environments. It is home to a number of endangered and threatened species including ospreys and bald eagles. Previous analyses of environmental samples collected quarterly showed limited transport of DU within the impact area and no transport to the installation boundaries (Oxenberg, 1997). These results contrasted transport model predictions and suggested that chemical, physical, and/or biological processes may limit contaminant transport in local subsurface environments (Chen and Yiacoumi, 2002).

In ambient conditions, zero-valent DU is thermodynamically unstable (Grenthe et al., 1992) and will eventually be oxidized to water-soluble uranyl ions (UO_2^{2+}) and other aqueous species via interaction with air and moisture. The initial oxidation of UO_2^{2+} involves the following reactions:

$$U(s) + O_2(g) \Leftrightarrow UO_2(s) \quad (1)$$

$$U(s) + 2H_2O \Leftrightarrow UO_2(s) + 2H_2(g) \quad (2)$$

$$UO_2(s) + \tfrac{1}{2}O_2(g) + 2H_2O \Leftrightarrow UO_3 \cdot 2H_2O(s) \quad (3)$$

$$UO_3 \cdot 2H_2O(s) + 2H^+ \Leftrightarrow UO_2^{2+}(aq) + 2H_2O \quad (4)$$

$$UO_2(s) + 2H^+ + \tfrac{1}{2}O_2 \Leftrightarrow UO_2^{2+}(aq) + H_2O \quad (5)$$

In addition to UO_2^{2+}, the occurrence of other aqueous U(VI) species of potential importance depends upon pH and other aspects of water chemistry. Additional species can include complexes with hydroxide and carbonate (e.g., $UO_2(OH)^+$ and $UO_2(CO_3)_3^{4-}$) as well as possible complexes with dissolved natural organic molecules, and other aquatic constituents (e.g., fluoride and calcium, dependent on local conditions). The mobility of U(VI) in soil is typically controlled by sorption and biological transformation processes, which are in turn influenced by the U(VI) speciation, sediment properties, and other water chemistry conditions. An important question for risk assessment and management at the APG is whether the water-soluble U(VI) species are immobilized in the local sediments by geochemical and biological processes or whether they migrate with the water flow and get discharged into the nearby Chesapeake Bay.

A number of studies have demonstrated that U(VI) can sorb to clay minerals (Turner et al., 1996), oxides and oxyhydroxides of iron (Hsi and Langmuir, 1985; Payne et al., 1996), and other oxide minerals (Payne et al., 1998). Sorption of inorganic U(VI) to individual minerals can be well described by surface complexation and ion-exchange models (Turner et al., 1996; Payne et al., 1998). In contrast, sorption of U(VI) from natural waters to soils and sediments is more complicated because of multiple sorption sites and the difficulty to unambiguously characterize either the full spectrum of aquatic species present or the individual properties and contribution to overall U(VI) sorption of the wide variety of co-mingled solid-phase sorbents, including natural organic matter (Davis et al., 2004).

Natural organic matter (NOM) exists in natural waters in stable colloidal and dissolved forms (mostly humic substances) and is also a major component of the solid phases in environmental soils and sediments. Humic substances can occur as deposits on oxides and clay minerals, where they

will form a surface coat that alters the mineral's relative affinity to metallic sorbates (Choppin, 1992). Because the DU test site at APG is a wetland area that features high concentrations of organic matter in both sediments and surface waters, it is expected that NOM plays an important role in controlling the behavior of uranium at the APG site with respect to both sorption of the heavy metal (Choppin, 1992; Payne et al., 1996; Zeh et al., 1997) and its bioavailability to microorganisms (Lovley et al., 1996; Gu and Chen, 2003).

Uranium-transforming microorganisms represent a potentially important determinant in the speciation and fate of the radionuclide at the site. Water-soluble U(VI) can be bioreduced and immobilized in anoxic conditions in the presence of organic matter via transformation to U(IV) by indigenous dissimilatory, metal-reducing bacteria (Lovley et al., 1991; Abdelouas et al., 1998; Fredrickson et al., 2002; Senko et al., 2002; Gu and Chen, 2003). Microbial reduction of U(VI) potentially can be accelerated significantly by the addition of fermentable electron donor compounds, such as acetate, ethanol and lactate (Lovley et al., 1991; Abdelouas et al., 1998). Furthermore, humic substances are known to play a beneficial role in some instances by serving as electron shuttles in uranium bioreduction (Gu and Chen, 2003). Overall, bioreduction of water-soluble U(VI) to insoluble U(IV) is an important mechanism for the immobilization of uranium in aquatic sediments and has been suggested as a method for the biological remediation of environments containing the toxic radionuclide (Lovley et al., 1991).

In this study, we explored the sorption and intrinsic bioreduction of uranium in APG sediment with the goal of better understanding the importance of both processes and their relative contribution to the immobilization of DU at the site. The experimental approach included abiotic sorption experiments in batch mode, geochemical computer modeling, anaerobic batch microcosms and non-culture dependent phylogenetic microbial profiling techniques to detect and identify microorganisms potentially involved in the bioreduction of uranium at the site.

2. Materials and methods

2.1. Collection, characterization and processing of environmental samples

Samples of two different sediments were collected aseptically from the Mosquito Creek Wildlife Refuge at the APG site (Aberdeen, MD, USA). The first sample (S1) was collected from the surface (0–15 cm) of a marshy area near the creek. The second sample (S2) was collected with a sterile trowel from the bottom of the creek. Samples were aseptically homogenized in the field, shipped to the laboratory on ice, and stored at 4 °C prior to further processing and analysis. Sample S1 was oven-dried in its entirety for 48 h at 110 °C, passed through a sterile 1-mm-mesh sieve, and stored at ambient temperature in the laboratory for later use in soil characterization and sorption experiments. A split sample of sediment S2 was treated as above. The remaining volume of sample S2 was placed in a sterile container and stored at 4 °C prior to construction of the microcosms.

Surface water (4 l) was collected aseptically from Mosquito Creek in a sterile glass jug and passed through a 0.22 μm cellulose acetate membrane (Fisher Scientific, PA) to remove bacteria. Inorganic chemistry information on surface water from the site was provided by a contract laboratory. Total organic carbon (TOC) dissolved in surface water was determined using a Phoenix 8000 UV-Persulfate TOC Analyzer (Teledyne Technol. Company, Mason, OH).

The weight percentages of sand, silt and clay were determined using Stock's sedimentation technique (Carter, 1993) following removal of natural organic matter (NOM) and free iron oxides (Klute, 1986). Sediment pH was measured in slurries prepared with distilled water (1:2 ratio) after 24 h of equilibration. The NOM content of individual samples was measured by weight loss upon ignition and baking at 500 °C for 6 h. Cation exchange capacity was measured using a Ba^{2+}/Mg^{2+} ion-exchange technique (Buurman et al., 1996). The free iron oxides were measured using a citrate–dithionite extraction method (Carter, 1993).

2.2. Preparation of U(VI) stock solution

Two U(VI) solutions were used. One contained 5 mg/l of uranium and was prepared from a plasma emission standard (1000 ppm uranium oxide in 5% nitric acid; Spectrum Chemicals, NJ) using sterile deionized water acidified to pH 1.5 via addition of HCl to ensure complete metal solubilization. Another solution containing 2.98 g/l of U(VI) was prepared for the batch sorption experiments by dissolving solid $UO_2(NO_3)_2 \cdot 6H_2O$ (Fisher Scientific, NJ) in deionized water that previously had been acidified to pH 1.5 via addition of HNO_3 to avoid precipitation. Stock solutions were used for spiking in sorption and bioreduction experiments. The nitrate concentration introduced by addition of stock solutions was estimated to be less than 10^{-4} mol/l.

2.3. Uranium analysis

Throughout the study, concentrations of U(VI) in solution were determined with a kinetic phosphorescence analyzer (KPA-11, Chemchek Instruments., Inc., Richland, WA) that was calibrated using commercial U(VI) standards (Spectrum Chemicals, New Brunswick, NJ). Since traces of chloride and dissolved organic matter can influence the measurement of U(VI) by KPA analysis, a wet-ash method was developed for this study to drive of hydrochloric acid and oxidize NOM. Aliquots (0.5–1 ml) of uranium solution were mixed with 2 ml of concentrated HNO_3 and 1 ml of 30% H_2O_2 solution. The mixture was boiled on a hot plate to near dryness at 100 °C in serum vials (Wheaton Science Products, NJ). The residual ash was resuspended in 0.1 N HNO_3 overnight and analyzed for U(VI). The effective instrument detection limit was determined to be 0.1 μg/l. All experimental glassware was cleaned using a mixture (1:1; v/v) of 30% H_2O_2 and 4 N HNO_3, followed by several rinses with sterile deionized water to remove any trace contamination of chlorine, organic matter or U(VI).

2.4. U(VI) sorption experiments

2.4.1. Kinetics of U(VI) sorption

Sorption of U(VI) to sediment material was studied at 22.5 °C in batch mode as a function of reaction time. Polyethylene bottles (1000 ml) used to determine the kinetics of U(VI) sorption to sediment S1 contained slurries consisting of 80 g of dried sediment suspended in 800 ml of sterile deionized water. Based on the results of preliminary experiments, samples were continuously agitated for 24 h to achieve hydration equilibrium. A volume of 800 μl of U(VI) stock solution (pH 1.5) was added and the introduced acidity was immediately neutralized with a small volume of 1 N NaOH solution. This protocol led to an initial uranyl ion concentration of 2980 μg/l. The mixture was agitated on a flatbed shaker (120 rpm) and aliquots of 1.5 ml were withdrawn periodically from the suspension using a syringe. Slurry aliquots were filtered through a 0.22 μm pore size membrane (Millipore-Millex, Bedford, MA) and the concentration of U(VI) remaining in the filtrate was determined by KPA analysis. The first 0.5 ml of filtered solution was discarded to minimize potential inaccuracies due to sorption of U(VI) to the filter membrane. The mass of sorbed U(VI) was calculated as the difference between the total U(VI) mass and the mass recovered from the aqueous phase. The soil-free control study indicated no loss of U(VI) to container walls and filters.

2.4.2. Sorption isotherm

A U(VI) sorption isotherm for sediment S1 was determined using initial U(VI) concentrations ranging from 238 to 23,800 μg/l. Sample S1 was first equilibrated in polyethylene centrifuge tubes (10 ml) using surface water without U(VI) at a solid/liquid ratio of 1:10. After 24 h of equilibration on a flatbed shaker, suspensions were centrifuged at 2000 × *g* for 30 min and the supernatants were spiked with stock solution to the desired U(VI) levels. Spiked samples were mixed and equilibrated in aerobic conditions at 22.5 °C for three days, a time period determined in preliminary kinetic experiments to be sufficient for reaching equilibrium. After equilibration was attained, suspensions were centrifuged at 2000 × *g* for 30 min and the supernatants were analyzed for pH and U(VI) concentration. Sorbed U(VI) mass was calculated as the difference between total U(VI) and the concentration of aqueous U(VI) at equilibrium. All experiments were conducted in triplicate.

2.4.3. Aqueous geochemical speciation modeling

A complexation model by Choppin and Allard (1986) was applied to calculate the speciation of U(VI) in surface water from the APG. Uranium complexation constants for the inorganic U(VI) ligands present in APG surface water (e.g., F^-, SO_4^{2-}, OH^-, and CO_3^{2-}) were obtained from the literature (Grenthe et al., 1992). Specific assumptions underlying the modeling efforts are discussed in detail in Section 3.

2.5. U(VI) bioreduction in sediment microcosms

2.5.1. Construction and incubation of sediment microcosms

Slurry microcosms were constructed in a glove box (Coy Laboratory Products, Inc., Grass Lake, MI) in media glass bottles (1.2 l) under the exclusion of oxygen (2% H_2 and 98% N_2) using 900 ml of surface water and 100 g of sediment. The added surface water was filter-sterilized (0.2 μm cellulose acetate filters; Millipore Corp., Bedford, MA) and spiked to an initial U(VI) concentration of 500 μg/l. Three different types of microcosms were constructed: (A) a sterile control slurry consisting of sterilized, U(VI)-spiked, surface water and sediment that was sterilized by gamma irradiation (4 Mrads) using a ^{60}Co source; (B) a non-sterile slurry microcosm (Live-1) simulating natural in situ conditions, constructed as above but with non-sterile sediment to study U(VI) bioreduction activity of indigenous microorganisms; and (C) another non-sterile slurry microcosm (Live-2), simulating enhanced in situ conditions, that was equivalent to Live-1 except for the addition of 38 mg of sodium acetate to stimulate accelerated bioreduction of uranium. Bottles were sealed with Teflon-lined butyl rubber septa and stirred with magnetic stir bars. Microcosms were incubated in a glove box in the dark at 22.5 °C. Periodically, 1.5 ml aliquots of the slurry were withdrawn, sediment was harvested by centrifugation at 2000 × *g* for 15 min, and the supernatant was used to measure U(VI) concentrations remaining in the aqueous phase. After decanting of the supernatant, bicarbonate buffer (0.1 M) was added to extract from sediment the mass of uranium that was deemed bioavailable. Carbonate-extractable uranium was assumed to be in the form of U(VI) sorbed to sediment (Senko et al., 2002). Residual extractable uranium, thought to represent the bioreduced U(IV) species, was extracted with nitric acid (1 M). Supernatant and extracts were treated using the above wet-ash method. The wet-ash residues were resuspended in 0.1 M HNO_3, and analyzed for U(VI) concentrations by KPA. Soluble and non-soluble phosphorus complexes were not considered because the concentration of phosphate in APG surface waters was negligible (phosphate concentrations $<10^{-5}$ mol/l).

2.5.2. DNA extraction

Crude DNA was extracted and purified from non-sterile microcosms with the FastDNA SPIN Kit for soil (Qbiogene, Inc., Carlsbad, CA) following the manufacturer's instructions.

2.5.3. Characterization of DNA

Enzymatic amplification of the 16S rDNA was performed on crude DNA extracted from the APG samples. Primers complementary to conserved regions among members of the domain *Bacteria* were used (8F and 1492R) as described previously (Lowe et al., 2002) to amplify a 550-bp fragment of the 16S rRNA gene corresponding to nucleotides 357–907 in the *Escherichia coli* sequence. The nucleotide sequence of the forward primer, which is specific for eubacteria (5′-CCTACGGGAGGCAGCAG-3′) contains at its 5′-end a 40-base GC clamp (5′-CGCCCGCCGCGCCCCGCGCCCGTCCCGCCGCCCCCGCCCG-3′) to stabilize the melting behavior of the DNA fragments. The reverse primer targeted the universal consensus sequence (5′-CCCCTCAATTCCTTTGAGTTT-3′).

2.5.4. Standard PCR reaction

PCR amplification was performed in a total volume of 50 μl in a 0.2-ml microfuge tube. Each tube contained 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris HCl (pH 9.0), 2% bovine serum albumin (BSA), 100 pmol each dNTP, 50 pmol of each primer and 1 unit of Redtaq genomic DNA polymerase (Sigma, MI). Template DNA at a concentration of 1 ng was added to the reaction mix. Samples were placed in a PCR machine (PTC-2000 DNA Engine Peltier Thermal Cycling System, MJ Research, MA). The reaction mix underwent a "hot start" where the temperature was increased to 94 °C for 5 min, followed by 20 cycles of 1 min at 94 °C, 1 min at 65–55 °C (touchdown-0.5 $cycle^{-1}$) and 1 min at 72 °C, followed by 10 cycles of 1 min at 94 °C, 1 min at 55 °C and 1 min at 72 °C, followed by a 7 min final extension at 72 °C. PCR products were reconditioned according to the method of Thompson (Thompson et al., 2002).

2.5.5. Analysis of PCR products by DGGE

To analyze the mixture of PCR fragments obtained by amplifications of the DNA extracted from the sampling wells, 50 μl of reconditioned PCR product was loaded onto the gel. Denaturing gradient gel electrophoresis (DGGE) was performed as previously described (Schafer and Muyzer, 2001) with 6% (wt/vol) acrylamide gels (in 0.5 TAE: 20 mM Tris acetate (pH 7.8), 10 mM sodium acetate, 0.5 mM disodium EDTA) containing a linear chemical gradient ranging from 35 to 60% denaturant. Gels were made with 6% (wt/vol) acrylamide stock solutions (acrylamide-*N*, *N*-methylene-bisacrylamide, 37:1) containing 0 and 100% denaturant (7 M urea and 40% [vol/vol] formamide, deionized with AG501-X8 mixed-bed resin [Bio-Rad Laboratories, Inc., Hercules, CA]). The gels were run for 18 h at 60 °C and 100 V.

2.5.6. Gel staining

DGGE gels were stained with ethidium bromide, using 10 ml of TAE buffer containing 50 μg ml^{-1} ethidium bromide. The tray was gently agitated for 15 min and then the solution was removed and replaced with distilled water and left for 10 min. The gel was visualized under a UV transilluminator using the gel documentation system (Alpha Imager 2000 V5.5; Alpha Innotech Corporation, VA).

2.5.7. Excision and sequencing of DGGE bands

Bands were excised from the gel using sterile pipette tips. The pieces of acrylamide containing the bands were incubated in 100 μl of sterile distilled water at 4 °C overnight. A 1-μl aliquot of this solution was used for PCR amplification. PCR products were re-run on the DGGE to confirm the purity of the band and the PCR product was purified with the QIAquick-spin DNA purification system (Qiagen, Valencia, CA) as per manufacturer's instructions. The cleaned PCR product was subjected to cycle sequencing.

2.5.8. Sequence alignment and analysis

The 16S rDNA sequences obtained from DGGE bands were edited using Chromas v1.45 (Technelysium, Helensvale, Australia) and assembled in Microsoft Word. Chimeric sequence analysis was performed using the Bellerophon server (Huber et al., 2004). Sequences found to be the result of chimera were discarded from further analysis. The nucleotide sequences were manually aligned according to the secondary structure of the 16S rDNA gene using Phydit software. Other 16S rDNA sequences with high similarity were obtained from GenBank using the BLAST family of programs (Altschul et al., 1990) and also included in the Phydit alignment. Of the 550 bp of sequenced 16S rDNA, approximately 330 bp were used for sequence alignment and tree construction. Evolutionary trees were generated using the neighbor-joining (Saitou and Nei, 1987), Fitch–Margoliash (Fitch and Margoliash, 1967), and maximum parsimony (Kluge and Farris, 1969) algorithms in the Phylip package (Felsenstein, 1989). Evolutionary distance matrixes for the neighbor-joining and Fitch–Margoliash methods were generated as described by

Jukes and Cantor (Jukes and Cantor, 1969). The confidence in tree topology was evaluated after 1000 bootstrap resamplings of the neighbor-joining data, and only values of 500 or higher were shown on the tree.

2.5.9. Sequence alignment and analysis

The GenBank accession numbers for the 16S rDNA sequences used to generate phylogenetic trees are as follows: *Clostridium subterminale* (AF241844), *Clostridium fallax* (AY208919), *Clostridium difficile* (X73450), Clone cD02014 (AJ617909), *Clostridium celerecrescens* (AJ295659), *Clostridium* sp. IrT-JG1-67 (AJ295658), *Clostridia* sp. X9Ba96 (AY607226), Clone IRR-DS5-29 (AJ621966), *Clostridium* sp. C4/1 (AY188850), Clone RB15 (U62838), *Clostridium tetani* (X74770), *Clostridium cylindrosporum* (Y18179), *Clostridium tunisiense* (AY187622), Clone cD484811 (AJ617911), *Anaerobacter polyendosporus* (AJ222546), *Clostridium acetobutylicum* (X78070), *Clostridia* sp. X9Ba90 (AY607220), *Clostridium* sp. Irt-JG1-9 (AJ295663), *Clostridium* sp. Irt-JG1-73 (AJ295661), Clone cD48722 (AJ617910), *Bacillus clausii* (AP006627), *Bacillus simplex* (AY833099), *Clostridium saccharobutylicum* (U16147), *Clostridium thermocellum* (L09173), Cyanobacterium clone (AY712382), Clone RCP2-13 (AF523902), *Cyanobacterium stanieri* (AF132782), *Synechococcus elongatus* (D83715), *Synecocystis* sp. 6803 (D90916), *Synechococcus* sp. 700246 (AF132775).

Sequences from bands excised from the DGGE gel comprising ~550 bp of the 16S rDNA gene were submitted to GenBank under the accession numbers AY919660–AY919666.

3. Results

3.1. Sediment and surface water characteristics

The two surface materials collected for the study represent two typical environmental conditions extant at the APG impact area. Material S1 originated from a location on the flood plain that is waterlogged for several months over the course of the year. It was characterized by a high sand content (~70%) with lesser amounts of silt and clay (19.1% and 3.2%, respectively). Sample S2 was obtained from the bottom of a creek that flows intermittently. It was predominantly composed of silt (~55%) with lesser amounts of sand and clay (17.6% and 15.7%, respectively). Aside from these differences, both materials, hereafter referred to as sediments, had similar characteristics (Table 1). They were relatively acidic (pH ~ 4) and rich in natural organic matter, with a NOM content of 6% and 10%, respectively. The acidic nature of the APG sediments can be attributed to the dissolution of humic and fulvic acids from the sediment, a process that is well documented in the literature (Schnitzer and Khan, 1972; Tepping, 2002).

The collected APG surface water at the site was also found to be acidic, with a measured pH value of 5.5, which is within the range of values recorded at the site (4.0–6.0) based on periodic monitoring. Dissolved organic carbon (DOC) content was measured to be 14 mg/l, and the principal detectable inorganic species were SO_4^{2-} (7.8–9.0 mg/l), F^- (0.11–0.31 mg/l), Cl^- (1.36–3.65 mg/l), HCO_3^- and CO_3^{2-}. Bicarbonate and carbonate concentrations were calculated to be equal to 1.8×10^{-6} and 4.9×10^{-11} mol/l, respectively, based on the average pH of 5.5 and assuming equilibrium with atmospheric CO_2.

Table 1
Selected properties of sediment samples S1 and S2 from the Aberdeen Proving Ground

Property	Unit	S1	S2
pH	Dimensionless	3.97	3.92
NOM	%	6.0	10.0
Free iron oxides	%	0.13	0.66
Cation exchange capacity	Millieq./100 g	1.3	2.4
Sand	%	69.9	17.6
Silt	%	19.1	54.7
Clay	%	3.2	15.7

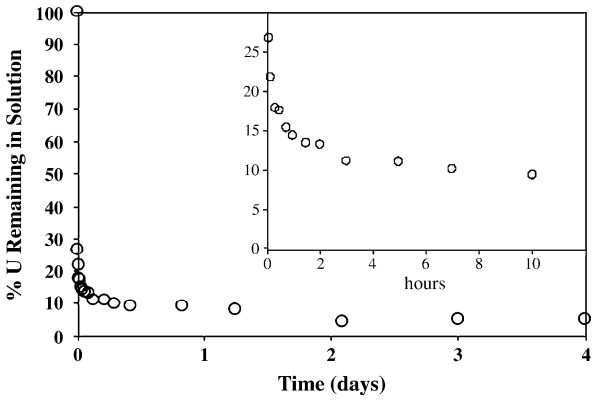

Fig. 2. Sorption of U(VI) as a function of reaction time in an aerobic, sterilized slurry mixed from surface water and S1 sediment (water-to-solid ratio of 10:1 ml/g) and spiked to an initial dissolved U(VI) concentration of 2980 μg/L (pH 4.8 ± 0.1; 22.5 °C).

3.2. Sorption and sorption kinetics of U(VI) in APG sediment

Sorption and sorption kinetics of U(VI) to APG sediment S1 were studied independently of biological processes in sterile, aerobic conditions. Sorption of U(VI) to sediment S1 was extremely rapid (Fig. 2). About 90% of the mass was lost from the dissolved phase within the first 3 h. After about 24 h, the system approached equilibrium, with approximately 5% of the mass remaining in solution at the end of the experiment on Day 4. The obtained sorption isotherm was linear with a slope approaching unity and a distribution coefficient, K_d, of 180 ± 5 ml/g (Fig. 3).

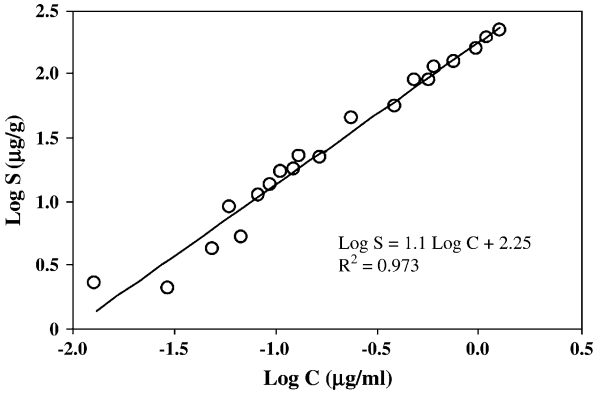

Fig. 3. Sorption isotherm for U(VI) in sterilized S1 sediment samples ranging in U(VI) concentration from 238 μg/L to 23,800 μg/L, incubated at pH 4.8 ± 0.1 and 22.5 °C in aerobic conditions using a water-to-solid ratio of 10:1 ml/g.

3.3. Speciation of uranium in surface water

Use of the complexation model by Choppin and Allard (1986) in conjunction with the measured dissolved organic carbon content and other measured chemical characteristics of the APG surface water resulted in the speciation diagram shown in Fig. 4. Further details on this modeling effort are provided below.

The speciation of U(VI) in surface water was calculated by considering the reactions of inorganic and organic ligands with U(VI). The chemical composition of surface water was as previously reported. Stability constants of U(VI) with the inorganic ligands were obtained from Grenthe et al. (1992) and corrected for the low ionic strength (~0.001 mol/l) of APG surface water using the Debye–Huckel equation.

When modeling metal speciation in natural waters, the major challenge is to accurately estimate the concentration of humic substances present in surface water. Since the molecular weight of humic substances is unknown and presumably has a wide range, its nominal concentration is generally expressed as the content of weakly acidic groups. In order to calculate the various uranium species in APG surface water under ambient conditions, the following assumptions were made: first, the concentration of humic substances can be estimated from the measured DOC concentration (14 mg/L) by applying a conversion factor of 1.73 (Zeh et al., 1997; Collins and Kuehl, 2001); second, the weakly carboxylic acid groups in humic substances are considered to act as the principal binding sites and their content was assumed to equal 1.5 mmol/g (Cronan and Aiken, 1985; Moulin et al., 1992); third, only the free carboxylic acid groups in humic substances were considered to form 1:1 and 1:2 complexes with UO_2^{2+} (Choppin and Allard, 1986) and fourth, the concentration of the free carboxylic groups was taken to be dependent on the degree of ionization of humic substances, which in turn was calculated as a function of pH.

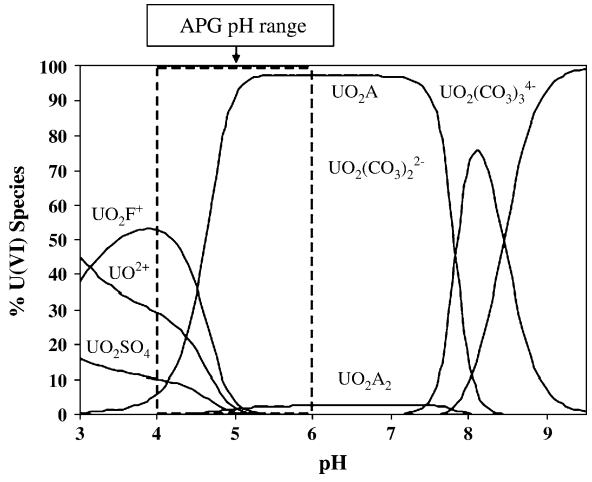

Fig. 4. Distribution of various U(VI) species in surface water ($I = 0.001$) in the presence of humic substances (DOC = 13.9 mg/l) as a function of pH at $P_{CO_2} = 10^{-3.5}$ atm, calculated using a humic acid complexation model published by Choppin and Allard (1986). The symbol A in UO_2A and UO_2A_2 represents free carboxylic acid groups in molecules of humic substances.

Based on these assumptions, the humic acid complexation model was used in conjunction with published inorganic complexation constants and our measured results to calculate the distribution of U(VI) species as a function of pH in surface water (Choppin and Allard, 1986) (Fig. 4). Overall trends in the distribution of U(VI) species are that (i) inorganic species dominate in highly acidic conditions (pH < 4), (ii) the humate species are the main carriers in the range of pH 4.5–7.5, and (iii) complexes of uranyl carbonate ($[UO_2(CO_3)_2^{2-}]$ and $[UO_2(CO_3)_3^{4-}]$) are the dominant ionic species in alkaline conditions (pH > 8.0). Note that humate complexes ($[UO_2A]$ or $[UO_2A(II)]$) are the major species of U(VI) in surface water at the APG in the ambient range of pH 4.6–6. The symbol A denotes the free carboxylic acid groups in humic substances. Nearly 100% of U(VI) is expected to be associated with humic substances at pH 5–7.5. Carbonate species of U(VI) dominate at pH ≥ 8. These speciation calculations suggest that the humate complex of U(VI) was the dominant species in the dissolved phase of our sorption and bioreducion experiments, for which pH was in the range of pH 4.8–5.5 after mixing and prolonged incubation.

3.4. Bioreduction of U(VI) in APG sediment slurries

Three different types of microcosms were used to elucidate the role of microorganisms in the fate of U(VI) in APG sediment (Table 2). Test systems were incubated in the dark at room temperature in anaerobic conditions, and periodically sampled over a period of 121 days. Similar to the results obtained with sediment S1 in abiotic conditions, rapid sorption of U(VI) to sediment S2 was observed in both the sterilized control and the two live microcosms (Fig. 5). The dissolved concentration of U(VI) decreased from 500 μg/l to 15 μg/l (97%) within hours. Thereafter, uranium concentrations in the aqueous phase remained stable in the sterile microcosm, whereas a slow further decline in concentrations was observed in the non-sterile systems. After 121 days, the aqueous phase of both biologically active microcosms contained about 5 μg/l of U(VI) (Fig. 5a).

An additional analysis of particle-associated uranium revealed a slow but steady bioreduction of U(VI) to U(IV) in both biologically active microcosms (Fig. 5b). Interestingly, this process would be very difficult (if not impossible) to detect by monitoring uranium concentrations in the aqueous phase only, owing to the insolubility of U(IV) (Fig. 5a).

Table 2
Composition of three types of aquatic microcosms constructed from surface water and S2 sediment from the Aberdeen Proving Ground (surface water-to-sediment ratio of 10:1; ml/g)

Microcosm ID	Sterilization by γ-irradiation	U(VI) added (μg)	Acetate (mg)
Live-1 (in situ conditions)	No	500	None
Live-2 (enhanced conditions)	No	500	38
Control	Yes	500	38

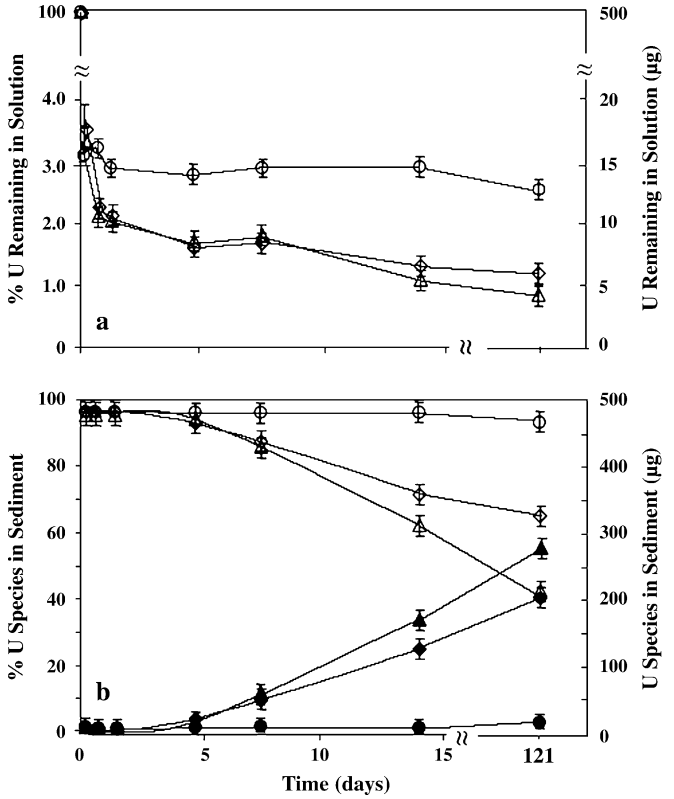

Fig. 5. Aqueous concentrations (a) and sorbed-phase concentrations (b) of U(VI) (open symbols) and U(IV) (closed symbols) in sterilized microcosms (○ and ●) and in non-sterile microcosms under anaerobic conditions and in the absence (◇ and ◆) and presence (△ and ▲) of 38 mg/kg acetate. In all systems, greater than 95% of U(VI) was removed from the aqueous phase almost instantaneously due to chemical sorption (a). Whereas U(VI) (○) and U(IV) (●) concentrations were essentially unchanged in the sterile microcosm, steady transformation of U(VI) (◇) to U(IV) (◆) was observed in the non-sterile microcosm (b). In the live microcosm containing acetate, biotransformation of U(VI) (△) to U(IV) (▲) was more extensive than in the absence of the electron donor compound following 121 days of incubation at ambient temperature (56% vs. 41% U(IV) final).

As shown in Fig. 5, essentially zero transformation of U(VI) occurred in the sterile control. In contrast, the U(VI) species was lost from non-sterile sediment (Live-1) in a linear process following zero-order kinetics. After 121 days of incubation, about 60% of uranium was carbonate extractable and presumably present as U(VI) (Senko et al., 2002) in the Live-1 microcosm simulating in situ conditions. The subsequent extraction of the sediment with nitric acid for recovery of U(IV) (Senko et al., 2002) yielded about 41% of the normalized total mass of uranium. Since the amount of U(IV) recovered from the sterile control was negligible, this finding suggests that uranium bioreduction occurred in APG sediment in the absence of external electron donors, resulting in the final conversion of 41% of the total radionuclide mass. In the acetate amended microcosm designed to simulate enhanced in situ conditions, only 44% of the total mass of uranium was detected in the form of U(VI). The balance of the mass was recovered by nitric acid extraction, suggesting that 56% of uranium had been bioreduced to U(IV).

3.5. Microbial community analysis

The denaturing gradient gel shown in Fig. 6 depicts the microbial assemblage of the two live microcosms after 12 days of incubation. This time period was deemed sufficient to allow for adaptation of the microbial assemblage to prevailing environmental conditions without risking complete and prolonged depletion of the added electron donor. Lanes 1 and 2 show, respectively, the profile of the two microbial communities under simulated in situ conditions and under conditions enhanced by the presence of acetate. The DGGE profile for the microcosm under in situ conditions is relatively simple, producing only 7 visible bands of similar intensity. Only two of these bands aligned well with bands from the enhanced, or acetate amended microcosm (dotted arrows). The DGGE profile from the acetate amended microcosm was more complex producing at least 11 different bands of varying intensities. Of these, four bands (#2, 3, 4 and 5) were significantly more intense than the others. These results indicate that the addition of acetate had a significant effect upon the bacterial community structure in the two live microcosms.

Bands unique to the acetate amended microcosm were excised from the denaturing gel and analyzed to infer the

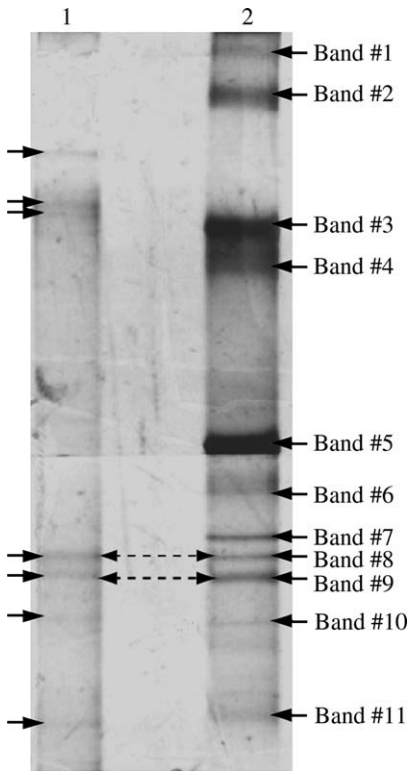

Fig. 6. DGGE separation pattern of bacterial DNA fragments coding for the 16S rRNA. Lane 1 corresponds to the live microcosm after 12 days of incubation in the absence of acetate (Live-1). Lane 2 corresponds to the live microcosm that received acetate prior to a 12-day incubation period (Live-2). Where indicated, bands were extracted and sequenced to infer the identity of bacterial species unique to the Live-2 microcosm. Dotted arrows indicate bands that are shared between the two microcosms.

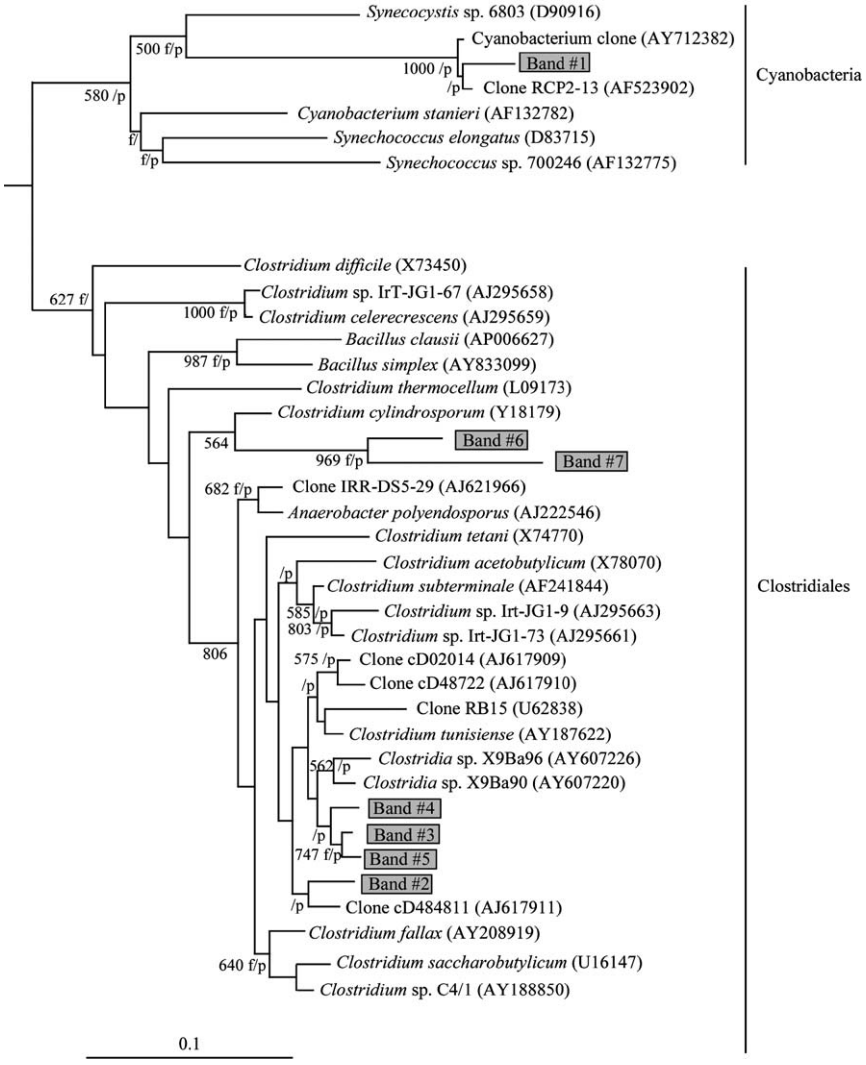

Fig. 7. Identification of bacteria unique to the acetate amended microcosm. A neighbor-joining tree was generated using ~330 bp of 16S rDNA excised from the denaturing gel shown in Fig. 6. The resulting tree shows the relationships between bacterial 16S rDNA sequences unique to the acetate amended microcosm, and sequences obtained from the GenBank database. The GenBank accession number is indicated in parentheses after each species or clone name. Bootstrap values ($n = 1000$ replicates) are indicated for the neighbor-joining method where values are greater than 500. An '*f*' or '*p*' indicates where the Fitch and parsimony methods are in agreement with the neighbor-joining tree. The bar represents 0.1 units of evolutionary distance.

identity of the bacterial species present (Fig. 7). As shown in the phylogenetic tree, DNA sequences obtained from the most intense bands, #2, 3, 4 and 5 formed a loose clustering within the *Clostridiales* near other uncultured, or unidentified *Clostridium* species. Blast analysis showed that bands #4 and 5 were most similar to two unidentified 16S rDNA clones obtained from rice paddy soil (97% identity, 498 of 511 bp and 97% identity, 406 of 417 bp, respectively). Band #2 was most similar to *A. polyendosporus* (95% identity, 335 of 349 bp) and band #3 was most similar to an uncultured *Clostridium* species, also obtained from rice paddy soil (97% identity, 349 of 357 bp) (Lueders et al., 2004). Sequences excised from less intense bands #6 and 7 also clustered in the *Clostridiales* and grouped with *C. cylindrosporum* (Fig. 7). Band #6 was most similar to *C. cylindrosporum* (88% identity, 245 of 276 bp) while band #7 was most similar to an uncultured *Clostridium* species from PCB-contaminated sediment of the Hudson River (91% identity, 113 of 124 bp). Finally, band #1 was most similar to a 16S rDNA clone obtained from a coal-contaminated wetland and clustered within the cyanobacteria.

4. Discussion

4.1. The role of NOM in the fate of uranium in APG sediment

As previously discussed, the high NOM content of the APG sediment and surface water is expected to influence both the mobility of the radionuclide in the subsurface and its availability to biotransformation by naturally occurring, metal-reducing microorganisms. In this section, the role of NOM in controlling sorption at the APG site is further explored.

4.1.1. Linearity of sorption isotherm and implied K_d

Because biological activity was precluded in the sorption isotherm experiments (through the use of oven-dried sediments and filter-sterilized surface water) and because abiotic reduction of U(VI) to U(IV) was prevented (by conducting the experiments in aerobic conditions), the observed rapid loss of 95% of the mass of U(VI) from the aqueous phase (Fig. 2) must be attributed exclusively to abiotic sorption. Considering 24-h sorption data, a sorption isotherm was obtained, for U(VI) with S1 sediment at 22.5 °C (Fig. 3). These data were fit to the Freundlich equation, which states in its logarithm form that:

$$\mathrm{Log}\, S = \mathrm{Log}\, K + n\, \mathrm{Log}\, C, \tag{6}$$

where S (μg/g) represents the concentration of U(VI) sorbed to the solid, C (μg/ml) denotes the aqueous concentration of U(VI) after sorption, and K and n represent characteristic constants. Experimentally determined values of Log K and n in sediment S1 were 2.25 ± 0.05 and 1.1 ± 0.1, respectively. The value of n is approaching unity, reflecting that sorption of U(VI) to S1 was essentially independent of the initial U(VI) concentration over the range of concentrations evaluated in this study (238–23,800 μg/l). Under these conditions, the constant K in Eq. (6) represents the constant ratio of S to C, which often is referred to as the distribution coefficient, K_d. For the APG data, the average value of K_d is calculated as 180 ml/g. Using this information, the percentage of U(VI) sorption for any given water-to-solid ratio (V/m) can be calculated as follows:

$$\%U\ \text{sorbed} = \frac{K_d}{K_d + V/m}, \tag{7}$$

where V and m are the aqueous volume (ml) and solid mass (g), respectively. This equation thus reflects the fact that in the batch systems with 1 g solid in 10 ml water, 95% of uranium mass was particle-associated, with only 5% dissolved in the aqueous phase. Under groundwater conditions (where V/m is more in the range of 0.5–0.6), an even smaller fraction of uranium (<1%) would be expected to reside in the aqueous phase, where it would be readily available to microorganisms for bioreduction. Although this partitioning behavior does not necessarily preclude movement of uranium, it would most certainly slow migration of the contaminant, however.

4.1.2. Distribution of NOM between solid and dissolved phases

A dissolved organic carbon concentration of 172 ± 3 mg/l was measured in the experimental system of S1 sediment at a surface water ratio (1:10 g/ml). Less than 10% of this DOC came from APG surface water, which on average contained 13.9 ± 0.2 mg/l of DOC. Recognizing that the S1 sediment contains approximately 6% organic matter (Table 1), the fraction of NOM present in the solid phase (NOM_{sp}) of the experimental systems was calculated to be roughly 95%, as shown below:

$$\begin{aligned}\%\left(\mathrm{NOM_{sp}}\right) &= \frac{6.0\% \times 1000\ \text{mg} - 172\ \text{mg}/1000\ \text{ml} \times 10\ \text{ml} \times 1.73}{6.0\% \times 1000\ \text{mg}} \\ &= 95\%, \end{aligned} \tag{8}$$

where 1.73 is a conversion factor used to translate dissolved organic carbon (DOC) into dissolved organic matter (DOM) (Zeh et al., 1997; Collins and Kuehl, 2001).

Because the mass of U(VI) sorbed was also about 95%, these findings suggest that NOM may have been the controlling factor for sorption of U(VI) in APG sediment. One possible explanation for these findings is that NOM associations account for the major fraction of U(VI) in both the aqueous and sorbed phases. In other words, deposited or mineral-bound humic substances may provide the major sink for U(VI) transiently passing through surface water. In this scenario, sorbed U(VI) would exist primarily in association with solid-phase humic substances (HS_{sp}) and aqueous phase U(VI) would exist primarily in associations with dissolved humic substances (HS_{aq}). This latter hypothesis is consistent with the hypothetical aqueous speciation diagram for this system (Fig. 4), which suggests that humic-complexed U(VI) is the dominant aqueous species at the pH of our experimental systems (pH 4.8). Conceptually, one may consider that the concentration of any freely dissolved inorganic U(VI) in the system, i.e. $U(VI)_{aq}$, will be subject to competition between the dissolved and solid-phase HS according to the following general reactions:

$$\mathrm{U(VI)} + \mathrm{HS_{sp}} \Leftrightarrow \mathrm{U(VI){-}HS_{sp}}, \tag{9}$$

$$\mathrm{U(VI)} + \mathrm{HS_{aq}} \Leftrightarrow \mathrm{U{-}HS_{aq}}, \tag{10}$$

where $U(VI)-HS_{sp}$ represents the HS complex of U(VI) in the solid state and $U(VI)-HS_{aq}$ denotes the complex of U(VI) with dissolved HS in aqueous phase. Assuming identical complexation constants for $U(VI)-HS_{sp}$ and $U-HS_{aq}$, application of this model to our data suggests an equivalent distribution of U(VI) and NOM between the solid and aqueous phases.

4.2. The role of microorganisms in the fate of uranium in APG sediment

The microcosm experiments were designed to explore the potential biotransformation of U(VI) to the less soluble and less toxic species U(IV), according to the reaction:

$$\mathrm{UO_2^{2+}} + 2e^- \,(\text{from NOM or acetate}) \Rightarrow \mathrm{UO_2(s)}, \tag{11}$$

in which the electrons (e^-) may originate from either NOM or an added electron donor compound, in this case acetate. At 97%, initial abiotic sorption of U(VI) to sediment S2 was slightly more extensive than previously observed with sediment S1, which probably was due to the relatively higher NOM content of sediment S2 (see Table 1). The discrepancies

observed over time between the control and the two live microcosms must be attributed to the activity of indigenous microorganisms that rapidly began to bioreduce U(VI) to U(IV) (Fig. 5). Although this process did not require external electron donors, the addition of acetate assisted in achieving a slightly faster and more extensive bioreduction of U(VI).

The nitric acid extractable mass of U(IV) increased from 4% to 41% in the absence of acetate in simulated in situ conditions. This process likely was driven by biologically induced electron transfer from NOM to U(VI) according to Eq. (11), as described previously by others (Gu and Chen, 2003). The nitric acid extractable uranium increased from 3% to 56% in the presence of acetate, demonstrating that the intrinsic bioreduction rate is limited by the availability of an electron donor and potential carbon source, i.e. acetate.

Natural organic matter and the humic substances contained therein are known to be redox reactive and, therefore, capable of reducing redox-sensitive metals such as Fe(III), Cr(VI) and U(VI) (Lovley et al., 1996; Lovley and Blunt-Harris, 1999; Gu and Chen, 2003). Lovely postulated that humic substances likely were acting as electron shuttles between microorganisms and Fe(III) or its oxides (Lovley et al., 1996). Most recently, Gu and Chen (2003) found that various forms of NOM, particularly the soil humic acid could effectively enhance the bioreduction of U(VI) in the presence of *Shewanella putrefaciens* CN32 under anaerobic conditions. The results shown in Fig. 5 suggest, but do not confirm, that NOM contained in sediment from the APG also can effectively drive the bioreduction of U(VI) to U(IV).

The acetate-induced shift in the microbial community structure as shown in Fig. 6 resulted in the appearance of new, apparently dominant members phylogenetically related to the *Clostridiales* (Fig. 7, bands #2, 3, 4 and 5). The shift in community structure between the two microcosms was associated with an increased rate of U(VI) reduction suggesting that *Clostridium* species were involved in the bioreduction process. *Clostridium* species are obligate anaerobic, low G + C Gram-positive rods defined by their ability to form endospores and their lack of dissimilatory sulfate reduction. They are ubiquitous in soils, lakes and in the intestinal tracts of mammals. Gram-positive bacteria have been shown to reduce U(VI) including several sulfate reducing bacteria such as *Desulfosporosinus* sp. (Suzuki et al., 2004), the radiation resistant bacterium, *Dienococcus radiodurans* (Fredrickson et al., 2000) and *Clostridium* spp. In particular, *Clostridium* spp. have been shown to reduce U(VI) in both pure cultures and environmental samples. Francis et al. (1994) found that a *Clostridium* sp. ATCC 53464 can reduce U(VI) to U(IV) and possibly further to U(III). This process occurred enzymatically since spent medium did not cause U(VI) reduction following sterilization. Recently, Suzuki et al. (1997) showed that *Clostridium* sp. is one of several major contributors to U(VI) reduction in uranium mine sediments. It is thought that the reduction of U(VI) by *Clostridia* may occur naturally through the production of hydrogen and other fermentative end products. Given this information, it is not surprising that in this study we found *Clostridium* sp. to be the initial, major bacterial species associated with an increased rate of U(VI) reduction when a fermentable carbon source (i.e., acetate) was provided in APG sediments. It is important to note that at higher concentrations of U(VI) (>500 ppb), or when incubated for longer durations, other common U(VI) reducers such as *Geobacter* or *Desulfovibrio* species may become dominant as has been shown by others (Anderson et al., 2003).

5. Conclusions

Our laboratory studies demonstrated that abiotic sorption to sedimentary NOM and intrinsic bioreduction of uranium by indigenous microorganisms play a key role in the immobilization and fate of uranium in surface sediments at the APG. When extrapolated to in situ conditions, these results suggest that the two processes may constitute a protective mechanism potentially slowing the migration of uranium from the military test area into the Chesapeake Bay. However, this mechanism may shield the Bay only in normal meteorological conditions and stable redox conditions. During extreme climate events such as heavy rainfall, both uranium species will be susceptible to erosion and flushing into the estuary even when tightly bound to NOM and other particulates present at the site. In addition, reoxidation of U(IV) to U(VI) potentially may lead to remobilization of the heavy metal and radionuclide. However, the results from this study produced no evidence suggestive of substantial transport of U(VI) at the APG site.

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Disclaimer

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