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Microbial Reduction of U(VI) at the Solid−Water Interface

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Microbial (*Geobacter sulfurreducens*) reduction of 0.1 mM U(VI) in the presence of synthetic Fe(III) oxides and natural Fe(III) oxide-containing solids was investigated in pH 6.8 artificial groundwater containing 10 mM $NaHCO_3$. In most experiments, more than 95% of added U(VI) was sorbed to solids, so that U(VI) reduction was governed by reactions at the solid−water interface. The rate and extent of reduction of U(VI) associated with surfaces of synthetic Fe(III) oxides (hydrous ferric oxide, goethite, and hematite) was comparable to that observed during reduction of aqueous U(VI). In contrast, microbial reduction of U(VI) sorbed to several different natural Fe(III) oxide-containing solids was slower and less extensive compared to synthetic Fe(III) oxide systems. Addition of the electron shuttling agent anthraquinone-2,6-disulfonate (AQDS; 0.1 mM) enhanced the rate and extent of both Fe(III) and U(VI) reduction. These findings suggest that AQDS facilitated electron transfer from *G. sulfurreducens* to U(VI) associated with surface sites at which direct enzymatic reduction was kinetically limited. Our results demonstrate that association of U(VI) with diverse surface sites in natural soils and sediments has the potential to limit the rate and extent of microbial U(VI) reduction and thereby modulate the effectiveness of in situ U(VI) bioremediation.

Introduction

U(VI) typically exists as soluble carbonate complexes (e.g., $UO_2(CO_3)_2^{2-}$ and $UO_2(CO_3)_3^{4-}$) in circumneutral pH groundwaters, and the formation of such complexes enhances U(VI) mobility in the subsurface (*1*). In contrast, uranium in the +4 oxidation state forms sparingly soluble minerals such as uraninite (UO_2(s)) (*2*). Biological U(VI) reduction by dissimilatory metal reducing bacteria (DMRB) is currently recognized as a promising strategy for in situ remediation of uranium-contaminated subsurface environments in which Fe(III) oxides are abundant electron acceptors for DMRB growth and maintenance (*3*−*7*).

Although soluble U(VI)-carbonate complexes are common in circumneutral pH groundwaters, such complexes (as well as the uranyl ion) are subject to adsorption onto Fe(III) oxide and other mineral surfaces (*8*−*13*). Thus, complexation of U(VI) by mineral surfaces is likely to play an important role in governing the behavior of uranium in subsurface environments, even in the presence of substantial levels of dissolved inorganic carbon. Desorption of U(VI) from mineral surfaces (e.g. in highly contaminated source zones) represents a potential long-term source of uranium input to groundwaters (*14*), analogous to the slow release of chlorinated hydrocarbons from DNAPL source zones (*15*). An important unanswered question with regard to U(VI) reduction by DMRB is whether sorbed U(VI) is subject to efficient reduction, as is the case for aqueous U(VI) species. This question is significant with respect to the design of in situ U(VI) bioremediation procedures, because if sorbed U(VI) can be rapidly and efficiently converted to an immobile U(IV) phase (i.e. UO_2(s)), then stimulation of DMRB activity in source zones may provide a effective means for preventing long-term subsurface contaminant migration. In addition, the efficacy of in situ biogenic redox barriers for prevention of far-field U(VI) migration in relatively dispersed plumes (*16*) would be enhanced if the U(VI) that entered and underwent surface complexation in such zones was subject to rapid and complete microbial reduction.

The purpose of this research was to investigate the potential for reduction of U(VI) associated with solid-phase mineral surfaces by a representative DMRB (*Geobacter sulfurreducens*). A parallel study evaluated the potential for abiotic U(VI) reduction by sorbed Fe(II) (*17*), with the goal of determining whether biotic or abiotic processes are likely to dominate U(VI) reduction activity in circumneutral pH groundwater environments supporting DMRB activity. Key to both of these investigations was the comparison of U(VI) reduction in suspensions of synthetic Fe(III) oxides vs natural Fe(III) oxide-bearing solids, which revealed the potential influence of sorption site heterogeneity on biotic and abiotic U(VI) reduction.

Experimental Section

Microorganism and Culture Conditions. The DMRB *Geobacter sulfurreducens* was obtained from the American Type Culture Collection (ATCC 51573). This organism, originally isolated from hydrocarbon-contaminated soil, is an anaerobic respiratory Gram-negative bacterium capable of growth with reduction of Fe(III) and other oxidized metals as electron acceptors (*18*, *19*). *G. sulfurreducens* was maintained at 30 °C in bicarbonate-buffered (30 mM $NaHCO_3$; 80% N_2/20% CO_2 headspace) acetate/ferric citrate medium (50 mM ferric citrate, 10 mM CH_3COONa) supplemented with inorganic nutrients (4.4 mM KH_2PO_4, 28 mM NH_4Cl) and vitamin and trace mineral solutions (*20*). To obtain large quantities of cells for Fe(III)/U(VI) reduction experiments, *G. sulfurreducens* was grown for three generations in medium with fumarate (50 mM) as the electron acceptor. Cells from third-generation fumarate cultures were harvested by centrifugation (7000 rpm, 15 °C) and washed twice with sterile, anoxic Pipes (1,4-piperazine-*N*,*N*′-bis-2-ethanesulfonic acid) buffer (10 mM, pH 6.8). The washed cells were resuspended in Pipes buffer in an anaerobic chamber (Coy Laboratory Products, Inc., Grass Lakes, MI) under a N_2:H_2 (ca. 98:2.0) atmosphere,

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TABLE 1. Characteristics of Synthetic Fe(III) Oxides and Natural Fe(III) Oxide-Bearing Materials

oxide	C/D Fe(III)[a] (μmol g^{-1})	0.5 M HCl Fe(III)[b] (μmol g^{-1})	HClFe(III)/ C/D Fe(III)	surface area (m^2 g^{-1})[c]	surface area (m^2 L^{-1})[d]	microporosity (μL g^{-1})[e]	microporosity (μL L^{-1})[f]	dominant Fe(III) oxide phase (ref)
synthetic goethite	11139 ± 323	131 ± 91	0.0118	55.2	247	ND[g]		goethite (*55*)
synthetic hematite	12333 ± 1874	99.3 ± 14.1	0.0081	9.0	46.5	0.68	2.8	hematite (*22*)
APS	520 ± 17	18.8 ± 1.0	0.0361	45.3	4356	5.35	514	goethite (*23*, *24*)
OS	825 ± 18	48.0 ± 4.2	0.0581	64.3	3897	9.15	555	goethite (*23*, *24*)
FRC	345 ± 41	29.6 ± 8.6	0.0794	31.0	4492	4.46	646	goethite (*56*)
NH	6300 ± 168	56.2 ± 16.1	0.0089	25.7	204	3.19	25.3	hematite (*28*)
NG	3143 ± 150	8.5 ± 0.4	0.0027	27.1	431	ND		goethite (*29*)
CP	574 ± 47	6.8 ± 1.7	0.0118	15.9	1387	2.29	200	goethite (*25*)
HC	691 ± 47	2.8 ± 0.1	0.0042	19.0	1375	ND		hematite (*25*)
Paleosol	140 ± 34	11.3 ± 2.0	0.0806	19.3	6893	ND		hematite (*27*)

[a] 1-h extraction with 0.2 M sodium citrate/0.35 M acetic acid + 50 mg mL^{-1} sodium dithionite, pH 4.8; mean ± SD of triplicate determinations, corrected for 0.5 M HCl-extractable Fe(II). [b] 1-h extraction with 0.5 M HCl; mean ± SD of triplicate samples, corrected for 0.5 M HCl-extractable Fe(II). [c] Determined by multipoint BET N_2 adsorption (Micromeritics Model Gemini). [d] Equal to BET surface area times the mass of material per L of suspension. [e] <20 Å; determined by N_2 adsorption (Micromeritics Model ASAP 2000 Porosimetry Analyzer). [f] Equal to microporosity times the mass of material per L of suspension. [g] Not determined.

after which the cell density was determined by measuring the absorbance at 600 nm.

Fe(III) Oxide Phases. Synthetic hydrous ferric oxide (HFO) was produced by adjusting 0.4 M $FeCl_3 \cdot 6H_2O$ to pH 7 with 1 M NaOH at room temperature. Synthetic medium surface area (ca. 55 m^2 g^{-1}) goethite (α-FeOOH) was produced by adjusting 0.4 M $FeCl_3 \cdot 6H_2O$ to pH 13 with 4 M NaOH and incubating the suspension at 70 °C for 16 h (*21*). The HFO and goethite were washed by centrifugation until the Cl^- concentration was < 0.5 mM. The goethite was freeze-dried and passed through a 100 μm sieve, whereas the HFO was stored as a hydrous gel at 4 °C. Hematite (α-Fe_2O_3) was purchased from J. T. Baker. X-ray diffraction analysis and Mössbauer spectroscopy showed that the hematite was greater than 99% pure (*22*).

Several previously characterized Fe(III) oxide-containing natural materials were used in this study, including (i) Pliocene and Pleistocene Age Atlantic coastal plain sediments (Abbott's Pit Sand, designated APS; and Oyster Sand, designated OS) collected from gravel pits (APS) or sediment cores (OS) on the Delmarva Peninsula, Virginia, U.S.A. (*23*, *24*); (ii) Fe(III) oxide/layered silicate mixtures obtained from Ultisols in Tennessee (Holston/Cloudland, Typic Fragiudult; designated HC) and North Carolina (Cecil/Pacolet, Typic Hapludult; designate CP) (*25*, *26*); (iii) weathered saprolite (designated FRC) from the U.S. Department of Energy's Natural and Accelerated Bioremediation Research (NABIR) Field Research Center (FRC) at Oak Ridge National Laboratory (ORNL) in Tennessee, which consists mainly of clay and silt-size particles heavily coated with Fe-oxides and to a lesser extent Mn-oxides (*11*); (iv) a Paleosol obtained from the U.S. Department of Energy's Hanford site within the Pasco Basin (*27*); and (v) unconsolidated, sand textured, Pleistocene-age, Atlantic Coastal Plain sediments from Eatontown, New Jersey, which are highly enriched in hematite (designated NH for natural hematite) or goethite (designated NG for natural goethite) phases existing as grain coatings, discrete micro-precipitates, and intergrain cements (*28*, *29*). All the natural solids were wet-sieved (100 μm) and freeze-dried except for the Paleosol which was wet-sieved (2 mm) and air-dried. Selected characteristics of the solids are provided in Table 1; additional properties of these materials are reported in the references listed above. The majority of the Fe(III) oxide content of the natural materials was in the form of crystalline phases, as indicated by the ratio of 0.5 M HCl-extractable to citrate/dithionite (C/D)-extractable Fe(III), which ranged from ca. 0.003 to 0.081 (0.3 to 8.1%).

Fe(III)/U(VI) Reduction Experiments. A Pipes-buffered artificial groundwater (PBAGW) supplemented with vitamin and trace mineral solutions (*30*) was used for all microbial reduction experiments. The major element composition of PBAGW was as follows (mM): $CaCl_2$ (0.62), KCl (0.2), $MgCl_2 \cdot 6H_2O$ (0.25), and Na−Pipes (10.0). Sulfate was omitted in order to preclude the activity of sulfate-reducing bacteria, whose spores could potentially survive autoclaving. Small quantities of inorganic nutrients (10−50 μM KH_2PO_4, 100−500 μM NH_4Cl) were included in order to facilitate growth/maintenance of the DMRB. Synthetic or natural Fe(III) oxides were added to obtain a total Fe(III) concentration of 50 mmol L^{-1}. Sodium acetate (10 mM) served as the electron donor for all experiments.

The cultures (50 mL of medium in 100-mL serum bottles) were bubbled with O_2-free 100% N_2, capped with thick butyl rubber stoppers, crimp sealed, and autoclaved (121 °C, 20 min). U(VI) was added to a final concentration of 0.1 mM from a sterile, anoxic stock solution containing 1 mM uranyl-acetate (Spectrum, Gardena, CA) and 100 mM $NaHCO_3$; addition of U(VI) thus resulted in the addition of 10 mM $NaHCO_3$ to the medium. The pH of the suspensions was adjusted to 6.8 by adding small amounts of sterile 1.0 M HCl or 1.0 M NaOH without opening the culture bottles. Gas chromatographic measurements of the dissolved inorganic carbon (DIC) content of cultures (see ref *31* for details) analogous to those described in this paper showed DIC values of 8−9 mM (E. Roden, unpublished data). pH values increased only slightly during Fe(III) oxide reduction (to maximum values of 7.0−7.2), and speciation calculations (using MINEQL+ (*32*)) employing aqueous uranyl-carbonate and uranyl-phosphate stability constants from Grenthe (*33*) indicated that the aqueous phase remained undersaturated with respect to U(VI) mineral phases such as metaschoepite ($UO_3 \cdot 2H_2O$), β-$UO_2(OH)_2$, and $(UO_2)_3(PO_4)_2$ during the incubations. The amount of U(VI) added to the reaction systems was comparable, when normalized to Fe(III) oxide abundance, to the high range of uranium contamination present in subsurface sediments at the FRC site at ORNL (*34*). In some experiments, anthraquinone-2,6-disulfonate (AQDS, Sigma, St. Louis, MO) was added to a final concentration of 0.1 mM from a sterile anoxic stock solution. Washed *G. sulfurreducens* cells were added 30−60 min after addition of U(VI) to obtain a cell density of ca. 10^8 cells mL^{-1} (ca. 20 mg dry weight L^{-1}). The cultures were incubated statically at 22 °C in an anaerobic chamber, and concentrations of Fe(II) and U(VI) were monitored over time as described below.

U(VI) Desorption Experiment. An APS reduction culture in which U(VI) reduction had ceased at ca. 60% reduction was heat-killed to prevent further microbial activity. Aliquots of the suspension were diluted 1:10 into sterile anoxic PBAGW

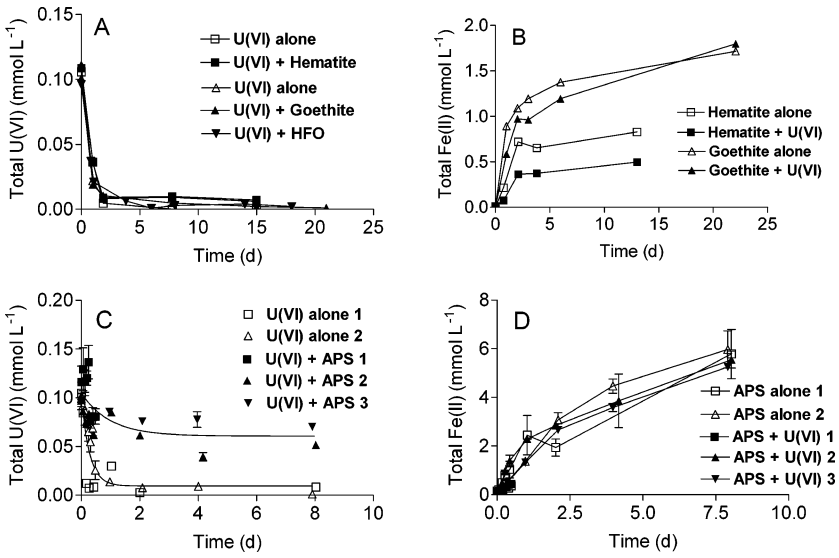

FIGURE 1. U(VI) reduction by *G. sulfurreducens* (10^8 cells mL^{-1}) in the presence or absence of 50 mmol Fe(III) L^{-1} in the form of either synthetic goethite, hematite, or HFO (panel A) or APS (panel C). Fe(III) reduction during these experiments (as well as in parallel uranium-free cultures) is shown in panels B and D. The labels 1, 2, and 3 in panels C and D correspond to separate experiments. Data points represent the means of duplicate cultures for panels A and B and triplicate cultures for panels C and D (error bars show ± 1 standard deviation). Lines in panel C are nonlinear least-squares regression fits of the data to the following equation: $C(t) = (C_o - C^*)\exp(-kt) + C^*$.

with or without 10 mM $NaHCO_3$, and the concentration of aqueous U(VI) in the diluted suspensions was followed over time.

Analytical Techniques. Samples for dissolved Fe(II) were filtered (0.2 mm syringe filter), and an aliquot of the filtrate was added to 5 mL of Ferrozine reagent (1 g L^{-1} ferrozine in 50 mM Hepes buffer) in the anaerobic chamber. After 10 minutes, the samples were removed, and the absorbance at 562 nm was determined with a Shimadzu UV-1201V spectrophotometer. Total Fe(II) was determined by adding a 0.5 mL aliquot of suspension to 5 mL of 0.5 M HCl. The solution was then removed from the anaerobic chamber and extracted on a rotary shaker for 1 h. The samples were filtered (0.2 mm) and analyzed using ferrozine. Dissolved and total U(VI) concentrations were analyzed with a Kinetic Phosphorescence Analyzer (KPA; Chemcheck Instruments, Richland, WA). Samples for dissolved U(VI) were filtered (0.2 mm) and acidified (0.01 N HNO_3 final concentration) in the anaerobic chamber. For total U(VI), a 1-mL aliquot of suspension was dispensed into 9 mL of anoxic 100 mM $NaHCO_3$, pH 8.4 (*35*), and the samples were extracted (under N_2) on a rotary shaker for more than 1 h. The samples were then filtered (0.2 μm) inside the anaerobic chamber, acidified, and analyzed with the KPA. Preliminary studies showed that the 100 mM $NaHCO_3$ extraction recovered 93.8 ± 7.2% (n=18), 95.8 ± 7.7 (n=18), and 98.6 ± 3.3% (n=12) of U(VI) added to sterile suspensions of synthetic goethite, hematite, and APS (50 mmol Fe(III) L^{-1}) over a 6-d incubation period.

X-ray Absorption Spectroscopy. U(VI) was added to APS and to NG with and without *G. sulfurreducens* (10^8 cells mL^{-1}), and the suspensions were incubated for 1 month. Total Fe(II) and U(VI) were then determined, and the suspensions were filtered (0.2 mm) in the anaerobic chamber. The solids retained on the filter were transferred to serum bottles. X-ray absorption spectroscopy (XAFS) data were collected at MR−CAT beamline (*36*) at the Advanced Photon Source at Argonne National Laboratory with a multielement detector in fluorescence mode. The percent of U(IV) was determined by using the U L_3 edge X-ray absorption near edge structure (XANES) data. All data sets were accurately aligned in energy using the derivative of the edge of a uranium phosphate standard that was measured simultaneously with the unknown samples as described elsewhere (*37*). The energy value at 0.5 edge step in the normalized absorption data was compared to the value for U(IV) and U(VI) standards. The accuracy of this procedure is conservatively estimated at 15%. Inhomogeneity of the samples gave rise to some variation in the percent U(VI) from successive scans (3 to 7) at different locations on the samples. The standard deviation from the values determined from each scan of each sample was added in quadrature to the 15% uncertainty estimate to determine the final uncertainty.

Results

Reduction of U(VI) in the Presence and Absence of Synthetic and Natural Fe(III) Oxides. More than 95% of added 0.1 mM U(VI) was sorbed within 3 h in suspensions of synthetic goethite, hematite, HFO, and the natural Fe(III) oxide-bearing APS and OS (data not shown). Rates of U(VI) reduction in the presence of the synthetic oxides were comparable to rates of aqueous U(VI) reduction observed in this (Figure 1A) and previous studies with different DMRB (*38*, *39*).

Rates of U(VI) reduction were significantly slower in the presence of APS and OS compared to the synthetic Fe(III) oxides, and a substantial fraction (40−60%) of the added uranium remained as sorbed U(VI) over a 1-month time period (Figures 1C and 2C). The presence of residual U(VI) in the APS suspensions was verified by XANES (Figure 3), which suggested that 65 ± 18% of the uranium was present as U(VI) in the microbially reduced materials, compared to 88 ± 15% in sterile unreduced controls. Rapid and complete reduction of U(VI) occurred upon addition of AQDS to *G. sulfurreducens* cultures, either at the start of the experiment (Figure 2A) or at a point in time when no further U(VI) reduction was occurring (Figure 2A,C). The results of these experiments are discussed in more detail below.

To confirm the results obtained with the Delmarva peninsula coastal plain sediments (APS and OS), six other natural Fe(III) oxide-bearing materials were used for U(VI) reduction experiments with *G. sulfurreducens* (Figure 4). For these experiments, Fe(III) reduction was allowed to proceed for 8−10 days prior to addition of U(VI) so as to facilitate comparison of biotic vs abiotic U(VI) reduction in parallel heat-killed controls as discussed elsewhere (*17*). Rapid and complete U(VI) sorption took place with all of the materials, except the Hanford Paleosol which sorbed only 35% of added

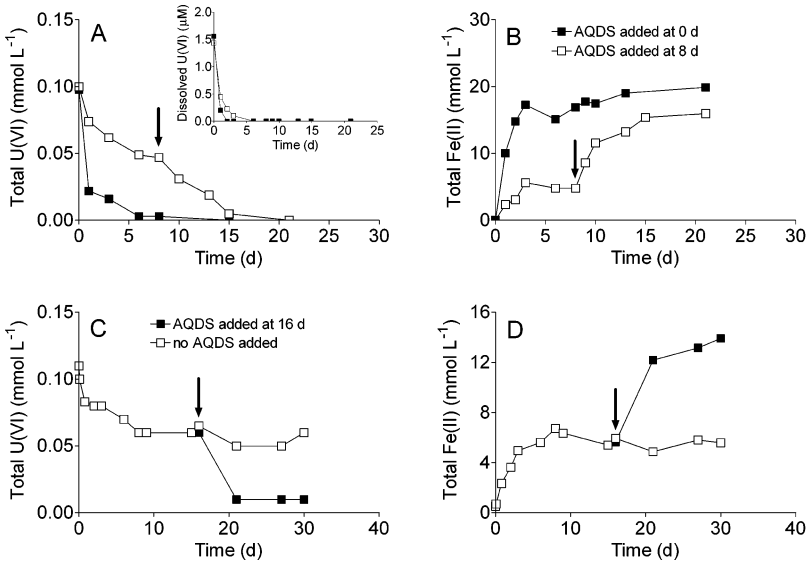

FIGURE 2. U(VI) and Fe(III) reduction by *G. sulfurreducens* (10^8 cells mL^{-1}) in APS (panels A and B) or OS (panels C and D) containing medium (50 mmol Fe(III) L^{-1}). The arrows indicate time at which 0.1 mM AQDS was added to cultures that did not initially contain AQDS. Data points represent the means of duplicate cultures. Inset in panel A shows change in dissolved U(VI) concentration over time.

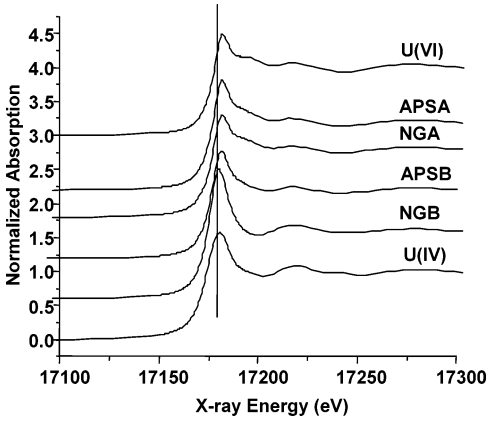

FIGURE 3. Averaged and normalized XANES data for microbially reduced (*G. sulfurreducens*, 10^8 cells mL^{-1}) and nonreduced APS and NG and U(IV) and U(VI) standards. Samples that show a mixture of U(IV) and U(VI) have a peak position between the U(IV) and U(VI) standards. APSA and APSB stand for APS abiotic and biotic samples, and NGA and NGB stand for NG abiotic and biotic samples, respectively. The percent U(VI) in the samples increased in the order NGB < APSB < NGA < APSA, with the values of 24 ± 16%, 65 ± 18%, 81 ± 19%, and 88 ± 15%, respectively.

U(VI) after 1 week of incubation (data not shown). A striking result of these experiments was the behavior of the suspensions of hematite- and goethite-enriched coastal plain sediment from Eatontown, NJ (NH and NG), in which rapid and extensive U(VI) reduction occurred within the first week of incubation (Figure 4A). These results are similar to those obtained in the synthetic Fe(III) oxide systems (Figure 1A), although the extent of U(VI) reduction in the NG suspension was lower than in the synthetic goethite system. XANES analysis confirmed the extensive reduction of U(VI) in the live NG suspension (Figure 3), indicating that 24 ± 16% of the uranium was present as U(VI) compared to 81 ± 19% in the sterile, unreduced control.

For all but one of the other suspensions of natural solids, rapid partial U(VI) reduction was followed by a period of slower reaction, leading to an asymptote of 60−80% reduction (Figure 4A). U(VI) reduction took place more gradually in the Paleosol suspension, which may have been due to formation of poorly reducible Ca−U(VI)-CO_3 complexes in the presence of abundant dissolved calcium (*40*) that originated from the solid-phase (the paleosol suspension contained ca. 2.5 mM dissolved Ca, compared to < 0.3 mM in the other suspensions). As observed in experiments with APS and OS (Figure 2A,C), rapid and near-complete U(VI) reduction occurred within 2 weeks after addition of AQDS to these systems (Figure 4C). In all cases, addition of AQDS also increased Fe(II) production (Figures 2B,D and 4B,D).

Discussion

U(VI) Reduction in the Presence of Synthetic versus Natural Fe(III) Oxides. This study demonstrated rapid and extensive reduction of U(VI) sorbed to synthetic Fe(III) oxides (60−70 wt % Fe) and highly Fe(III) oxide-enriched natural materials (18−35 wt % C/D-extractable Fe, see Table 1). The rates of U(VI) reduction observed in these experiments were comparable to those observed in parallel studies of abiotic Fe(II)-driven U(VI) reduction (*17*). Based on these results and the rapid production of Fe(II) in the synthetic Fe(III) oxide suspensions (Figure 1B), it is impossible to tell whether U(VI) reduction in these systems occurred via enzymatic activity, abiotic reaction, or a combination of both.

U(VI) reduction was generally slower and less extensive in the presence of natural soil or sediment materials with relatively low (intrinsic) Fe(III) oxide content (1−4 wt % C/D-extractable Fe) compared to more Fe-rich materials. The initial very rapid loss of ca. 10−20% of added U(VI) in the suspensions of microbially reduced natural materials (Figure 4A,C) was likely promoted by abiotic, Fe(II)-driven reduction (*17*). However, comparison with heat-killed systems indicated that continued reduction of U(VI) in the live systems was the result of ongoing DMRB activity (*17*). The ability of *G. sulfurreducens* to transfer electrons to U(VI) associated with sediment surfaces is consistent with the original study of Lovley et al. (*3*), which documented reduction of solid-associated (100 mM $NaHCO_3$ extraction) U(VI) by natural microflora in anaerobic aquatic sediments as well as more recent studies of U(VI) reduction by microorganisms in leachate-contaminated aquifer sediments (*14*, *41*).

The reason(s) for the incomplete reduction of U(VI) sorbed to natural Fe(III) oxide-bearing materials cannot be fully explained with available data. However, it seems likely that the association of U(VI) with enzymatically inaccessible micropores in the solids (or, more generally, surface sites at

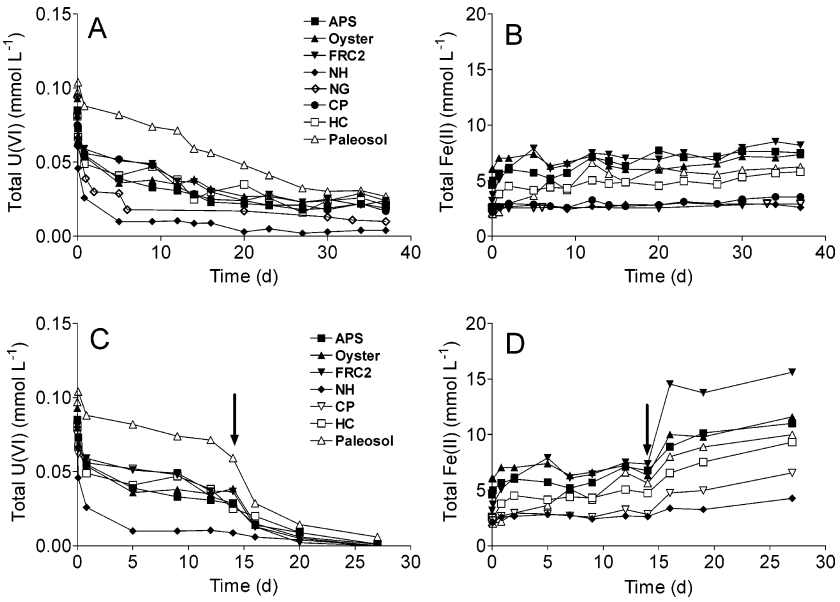

FIGURE 4. U(VI) and Fe(III) reduction by *G. sulfurreducens* (10^8 cells mL^{-1}) in natural Fe(III) oxide cultures with (panels C and D) and without (panels A and B) 0.1 mM AQDS. Fe(III) oxide reduction was allowed to proceed for 8−10 d prior to addition of U(VI). Arrow indicates time of AQDS addition. Data points show the mean of duplicate cultures.

which enzymatic reduction was sterically hindered) can account for the limited reduction of solid-associated U(VI) despite the presence of vigorous DMRB activity (as evidenced by ongoing Fe(III) oxide reduction). The natural materials had ca. 5 to 10-fold greater cumulative microporosity (<20 Å pore width) compared to the synthetic hematite (Table 1). Taking into account the mass loadings for the different suspensions indicates that the total microporosity present in the APS, OS, FRC, and CP suspensions was 1−2 orders of magnitude greater than in the synthetic hematite and NH suspensions (see Table 1).

There was weak but significant ($r^2 = 0.506$, $p = 0.048$, $n = 8$) inverse correlation between initial rates of U(VI) reduction (as estimated from nonlinear least-squares regression fits of the data in panel 4A to the generalized rate law discussed in refs *42*−*44*) and the amount of surface loading (in $m^2 L^{-1}$) for the different natural materials. This correlation supports the idea that association of U(VI) with sediment microporosity impeded U(VI) reduction. This argument also is supported by the effect of AQDS, which accelerated Fe(III) reduction and led to virtually complete U(VI) reduction in all cases (Figures 2 and 4). AQDS is known to function as an electron shuttle which can increase the rate and extent of both synthetic and natural Fe(III) oxide bioreduction (*7*, *28*, *29*, *45*−*49*). Since the reduction potential of the AQDS/AH_2DS couple at pH 7 (ca. −0.18 V (*50*)) is comparable to both (i) the midpoint potential of outer membrane-bound Fe(III) reductases in *G. sulfurreducens* (ca. −0.19 V (*51*)) and (ii) the equilibrium potential of *G. sulfurreducens* cells attached to graphite electrodes (ca. −0.17 V (*52*)), it seems likely that AQDS stimulated electron transfer to Fe(III) and U(VI) on kinetic rather than thermodynamic grounds, i.e., by shuttling electrons to oxide surface sites (e.g. ones located within micropores (*47*)) at which enzymatic electron transfer was kinetically limited. However, this remains only a tentative assertion, since we have no information on the effective reduction potentials of AQDS and Fe(III) reductases in our experimental systems, and since it is not yet known whether the Fe(III) reductase isolated by Magnuson et al. (*51*) is directly involved in Fe(III) oxide reduction (*53*), let alone reduction of U(VI) associated with mineral surfaces.

The observed stimulation of U(VI) reduction by AQDS contrasts with the results of Finneran et al. (*7*), who found that addition of AQDS stimulated reduction of Fe(III) but not aqueous U(VI) in uranium-contaminated aquifer sediments. The authors concluded that AH_2DS produced by DMRB activity reacted more rapidly with Fe(III) oxides compared to aqueous U(VI), such that U(VI) reduction was not enhanced by the presence of the electron shuttle. There is an important difference between this study and our experiments, however, in that Finneran et al. (*7*) examined reduction of soluble U(VI), whereas virtually all of the U(VI) in our systems was associated with solid surfaces. AQDS had minimal influence on reduction of the small amount of dissolved U(VI) initially present in the sediment suspensions (Figure 2A inset) but greatly stimulated reduction of sorbed U(VI). The simplest interpretation of these results is that AH_2DS reacted with U(VI) associated with enzymatically inaccessible surface sites. The ability of AH_2DS to reduce solid-associated U(VI) was independently demonstrated by experiments with heat-killed sediment suspensions (*17*). These results are consistent with the ability of AH_2DS to reduce the solid-phase U(VI) mineral metaschoepite (*6*).

A final issue pertinent to the persistence of U(VI) in the natural Fe(III) oxide-containing reduction systems is the apparent nonreversible association of U(VI) with surface complexation and/or ion exchange sites on the natural solids. This phenomenon is implied by the presence of residual solid-associated U(VI), because in the absence of it, enzymatic scavenging of U(VI) in the aqueous phase should have systematically drawn sorbed U(VI) off the solids and converted it to UO_2(s). Desorption experiments were conducted with APS reduction cultures in which U(VI) reduction had ceased at ca. 60% in order to assess whether the residual U(VI) was irreversibly bound. Substantial (ca. 55%) release of U(VI) into the aqueous phase was observed in $NaHCO_3$-containing PBAGW, whereas much less (ca. 15%) desorption occurred in $NaHCO_3$-free medium (Figure 5). Although quantitative interpretation of these results is beyond the scope of this paper, the degree of U(VI) desorption that occurred at pH and $NaHCO_3$ concentration identical to the original culture medium suggests that the residual U(VI) was not present in a permanent irreversibly bound state. Further studies of the speciation of residual sorbed U(VI) are required to evaluate the potential significance of this phenomenon for in situ U(VI) bioreduction.

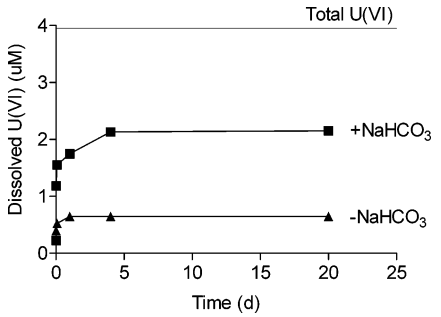

FIGURE 5. Desorption of residual U(VI) from pasteurized microbially reduced APS diluted 1:10 in PBAGW with or without 10 mM $NaHCO_3$. Data represent means of triplicate samples. The dashed horizontal line represents the total amount of U(VI) in the suspensions.

Environmental Significance. A key implication of our findings is that the association of U(VI) with surface sites on natural materials at which enzymatic reduction is limited may strongly modulate the effectiveness of subsurface U(VI) bioremediation. Although aqueous (mobile) U(VI) concentrations may decrease from μM-levels to much lower values during active bioremediation (as observed here; Figure 2A inset), the persistence of residual sorbed U(VI) on aquifer solids could lead to U(VI) release after the period of DMRB stimulation. Our results indicate that the presence of soluble electron-shuttling compounds may increase both the rate and extent of sorbed U(VI) reduction. These findings verify the predictions by Fredrickson et al. (*6*) and Finneran et al. (*7*) that the presence of AQDS should stimulate U(VI) bioreduction in Fe(III) oxide-reducing systems and indicate that addition of electron shuttling compounds, such as natural humic substances, could enhance the overall effectiveness of in situ U(VI) bioremediation. However, other recent studies indicate that humic complexation of U(VI) may inhibit microbial reduction of soluble U(VI) (*54*). Additional studies are required to evaluate the extent to which natural or synthetic electron shuttles may be able to facilitate U(VI) reduction in subsurface sediments.

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