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In situ mobility of uranium in the presence of nitrate following sulfate-reducing conditions

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ABSTRACT

Reoxidation and mobilization of previously reduced and immobilized uranium by dissolved-phase oxidants poses a significant challenge for remediating uranium-contaminated groundwater. Preferential oxidation of reduced sulfur-bearing species, as opposed to reduced uranium-bearing species, has been demonstrated to limit the mobility of uranium at the laboratory scale yet field-scale investigations are lacking. In this study, the mobility of uranium in the presence of nitrate oxidant was investigated in a shallow groundwater system after establishing conditions conducive to uranium reduction and the formation of reduced sulfur-bearing species. A series of three injections of groundwater (200 L) containing U(VI) (5 μM) and amended with ethanol (40 mM) and sulfate (20 mM) were conducted in ten test wells in order to stimulate microbial-mediated reduction of uranium and the formation of reduced sulfur-bearing species. Simultaneous push-pull tests were then conducted in triplicate well clusters to investigate the mobility of U(VI) under three conditions: 1) high nitrate (120 mM), 2) high nitrate (120 mM) with ethanol (30 mM), and 3) low nitrate (2 mM) with ethanol (30 mM). Dilution-adjusted breakthrough curves of ethanol, nitrate, nitrite, sulfate, and U(VI) suggested that nitrate reduction was predominantly coupled to the oxidation of reduced-sulfur bearing species, as opposed to the reoxidation of U(IV), under all three conditions for the duration of the 36-day tests. The amount of sulfate, but not U(VI), recovered during the push-pull tests was substantially more than injected, relative to bromide tracer, under all three conditions and further suggested that reduced sulfur-bearing species were preferentially oxidized under nitrate-reducing conditions. However, some reoxidation of U(IV) was observed under nitrate-reducing conditions and in the absence of detectable nitrate and/or nitrite. This suggested that reduced sulfur-bearing species may not be fully effective at limiting the mobility of uranium in the presence of dissolved and/or solid-phase oxidants. The results of this field study confirmed those of previous laboratory studies which suggested that reoxidation of uranium under nitrate-reducing conditions can be substantially limited by preferential oxidation of reduced sulfur-bearing species.
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1. Introduction

Uranium-contaminated groundwater is a human and environmental health concern due to releases associated with the mining, milling and processing of uranium ore as well as those from natural sources (Brugge et al., 2005). The mobility of uranium in groundwater is highly dependent on groundwater pH, redox potential and the mineralogy of the solid-phase subsurface media. In circumneutral pH groundwater, uranium primarily exists as soluble U(VI)-bearing species under oxidizing conditions or as less soluble U(IV)-bearing species under reducing conditions (Goodwin, 1982; Grenthe et al., 1992; O'Loughlin et al., 2011). Under oxidizing conditions and circumneutral pH, U(VI)-bearing species can be immobilized by adsorption to iron-bearing minerals (Li and Kaplan, 2012). Under reducing conditions, U(VI) can be reduced to immobile U(IV) chemically by reduced iron- or sulfur-bearing species (Chakraborty et al., 2010; Hyun et al., 2012, 2014; Jeon et al., 2005) and/or biologically by native anaerobic microbial communities (Wall and Krumholz, 2006). Microbial-mediated uranium reduction in particular, has been the predominant mechanism utilized for enhancing in situ uranium immobilization (Newsome et al., 2014). However, reoxidation of previously reduced uranium in the presence of dissolved- and/or solid-phase oxidants can result in remobilization of uranium, which poses a significant challenge for remediating uranium-contaminated groundwater (Singh et al., 2014).

Microbial-mediated reduction of uranium can be stimulated by the in situ addition of an electron donor such as ethanol, glucose, acetate, lactate, formate, or emulsified vegetable oil (Senko et al., 2002; Anderson et al., 2003; Istok et al., 2004; Vrionis et al., 2005; Wu et al., 2006, 2007, 2010; Dullies et al., 2010; Campbell et al., 2011; Sharp et al., 2011; Watson et al., 2013). In the presence of an added electron donor, uranium reduction can proceed following depletion of higher energy yielding terminal electron acceptors (TEAs) such as oxygen, nitrate, manganese, and concurrent with ferric-iron reduction (Newsome et al., 2014) which may result in the production of insoluble minerals such as uraninite (UO_2) (Wall and Krumholz, 2006). However, natural recharge of dissolved-phase oxidants such as oxygen and nitrate into previously reduced groundwater zones can result in reoxidation and subsequent remobilization of uranium (Wu et al., 2007, 2010; Watson et al., 2013). Although the presence of solid-phase oxidants such as Mn(IV)-oxides and/or Fe(III)-oxides can also result in reoxidation of uranium, their abundance is likely limited following uranium-reducing conditions (Vrionis et al., 2005). In order to actively maintain uranium-reducing conditions, the continuous or periodic addition of an electron donor can effectively prevent uranium reoxidation (Wu et al., 2007, 2010; Watson et al., 2013). However, active remediation systems can also be expensive to design, build, and operate. Therefore, creating groundwater conditions which can sustain uranium-reducing conditions after in situ electron donor addition has been terminated and depleted is of critical interest to remediation practitioners.

The importance of reduced sulfur-bearing minerals, formed by sulfate-reducing bacteria, has been recognized as a predominant factor contributing to maintaining uranium-reducing conditions in natural uranium-rich groundwater systems (Iwatsuki et al., 2004; Arthur et al., 2006; Noseck et al., 2012). This is likely due, in part, to preferential oxidation of common reduced sulfur-bearing minerals such as pyrite (FeS_2), mackinawite ($FeS_{0.9}$) and alabandite (MnS) by oxygen and nitrate, which are thermodynamically favorable reductants when compared to uraninite (Dean, 1999). This suggests that creating in situ groundwater conditions that are conducive to the formation of reduced sulfur-bearing minerals following uranium reduction may lead to greater stability of immobilized uranium in the presence of oxidants. The importance of preferential oxidation of reduced sulfur-bearing minerals following uranium reduction has been demonstrated experimentally in numerous laboratory studies (Abdelouas et al., 1999, 2000; Moon et al., 2009; N'Guessan et al., 2010; Bi et al., 2013; Bi and Hayes, 2014a, 2014b; Carpenter et al., 2015; Luan et al., 2015). For example, in a flow-through sediment column study, Moon et al. (2009) demonstrated that microbial-mediated uranium reduction followed by enhanced sulfate reduction resulted in the formation of iron sulfides which limited the extent of uranium reoxidation by oxygen and nitrate when compared to a previous study where uranium reduction was not followed by sulfate reduction (Moon et al., 2007). However, in both laboratory studies, the rate and extent of uranium reoxidation were greater when nitrate, as opposed to oxygen, was the oxidant. The relative importance of nitrate as a predominant oxidant for in situ uranium reoxidation has also been recognized at numerous uranium-contaminated sites where nitrate is a common co-contaminant due to activities associated with the processing of uranium ore (Spain and Krumholz, 2011; Lloyd and Renshaw, 2005; Smith et al., 2015). Although nitrate alone does not abiotically oxidize U(IV) to an appreciable extent, dissimilatory nitrate reduction intermediates, such as nitrite, nitric oxide, and nitrous oxide, as well as microbial-mediated nitrate-dependent U(IV) oxidation, have been shown to reoxidize uranium in numerous laboratory and in situ studies (Singh et al., 2014).

Despite the importance of nitrate as an oxidant under field conditions and sulfide-bearing minerals as reductants under laboratory conditions, relatively few studies to date have investigated uranium reoxidation by nitrate following sulfate-reducing conditions in the field. Therefore, a substantial knowledge gap currently exists as to the in situ feasibility of such an approach in terms of limiting the extent of uranium reoxidation. The objective of this study was to test the in situ mobility of uranium in the presence of nitrate following uranium- and sulfate-reducing conditions. Based on the results of previous studies and thermodynamics, we hypothesized that preferential oxidation of reduced sulfur-bearing species, as opposed to reduced uranium-bearing species, can substantially limit the extent of uranium mobilization in the presence of nitrate.

2. Materials and methods

2.1. Study site

The study site is located in Area 2 of the Oak Ridge Integrated Field Research Challenge (OR-IFRC) site in Oak Ridge, Tennessee. A typical geologic profile of Area 2 would consist of approximately 6 meters of reworked fill and saprolite at the surface underlain by 2 meters of intact saprolite with weathered bedrock below the saprolite (Watson et al., 2004).

The study site contains ten shallow groundwater monitoring wells (FW218 through FW227) constructed of ¾-inch inside diameter schedule 80 polyvinyl chloride (PVC) pipe. The monitoring wells were installed by direct push and are screened from 3.5 to 6 meters below ground surface (mbgs). The shallow groundwater aquifer is unconfined and depth to groundwater is approximately 3.5 mbgs. The groundwater and sediments within Area 2 are contaminated with nitrate and uranium from the former S-3 Ponds which contained liquid waste derived from the processing of uranium ore (Spain and Krumholz, 2011). The pH of groundwater at Area 2 tends to be between 6 and 7 with concentrations of uranium ranging from 3.8 to 7.1 μM (Moon et al., 2006) and concentrations of nitrate ranging from 1 to 4 mM (Spain and Krumholz, 2011). The average groundwater redox potential is 170 mV and reduction of equilibrium-predicted U(VI)-bearing species (UO_2CO_3, $UO_2(CO_3)_2^{2-}$, UO_2SO_4, $UO_2(SO_4)_2^{2-}$, $Ca_2UO_2(CO_3)_3$, $CaUO_2(CO_3)^{2-}$) is not energetically favorable in the absence of an added electron donor (Moon et al., 2006; Watson et al., 2013). The saprolite contains significant quantities of iron oxides and, to a lesser extent, manganese oxides which have a high capacity for U(VI) adsorption at circumneutral pH (Barnett et al., 2002). Concentrations of uranium (nitric-acid extractable) in saprolite from Area 2 range from 0.293 to 453 mg/kg (Moon et al., 2006). Microbial-mediated uranium reduction has been demonstrated in numerous laboratory studies utilizing Area 2 groundwater and/or sediments by the addition of a range of electron donors (Spain and Krumholz, 2011) and under in situ conditions by the addition of ethanol (Fang et al., 2006) and emulsified vegetable oil (Watson et al., 2013). However, concurrent reoxidation of reduced sulfur-bearing species and U(IV) by nitrate following depletion of emulsified vegetable oil has also been observed in Area 2 (Watson et al., 2013). This suggests that the geochemistry of Area 2 is conducive to forming reduced sulfur-bearing species but that nitrate has the oxidative strength to remobilize uranium to background levels. The wells utilized in this study were not part of any previous studies and are likely not affected by previous or ongoing activities within Area 2.

2.2. Biostimulation and reoxidation tests

A series of four tests were conducted in wells FW218 through FW227. Three biostimulation tests (tests 1, 2, and 3) were conducted in order to reduce and immobilize uranium and to precipitate sulfides (Table 1).

The reoxidation test (test 4) was conducted in order to investigate the mobility of uranium in the presence of nitrate (Table 1). Groundwater samples for all tests were collected and filtered (0.2 μm) in the field and stored at 4 °C until analyzed. Groundwater used for test injectate was collected from nearby well GW835 which contained relatively low pre-test concentrations of nitrate (1 mM), U(VI) (5 μM) and sulfate (1 mM), and a circumneutral pH (6.5) (Table 2). The test wells contained roughly similar pre-test concentrations of nitrate (0.1 to 12.9 mM), U(VI) (0.1 to 3.9 μM) and sulfate (0.1 to 1.9 mM) and a circumneutral pH (6.6 to 8.0) (Table 2). Pre-test concentrations of ethanol were below the method detection limit from injectate well GW835 and test wells FW218 through FW227 (data not shown).

The biostimulation tests were conducted by injecting 200 liters of ethanol- and sulfate-amended injectate in all ten wells (Table 1). Immediately prior to injection, the injectate was amended with 40 mM ethanol (C_2H_6O) and 20 mM sulfate (Na_2SO_4) and then mixed with compressed 80%N_2:20%CO_2 gas. The injectate was then injected into each well using a siphon and was completed within a 24-hour time frame. Five samples of the injectate were collected during injection for analysis of amended ethanol and sulfate (data not shown) and were similar to the target concentrations (Table 1). Groundwater concentrations of ethanol and sulfate in test wells immediately prior to subsequent biostimulation tests (data not shown) were similar to pre-test concentrations (Table 2).

The reoxidation test was conducted using the single-well push-pull test method according to the methodology of Istok et al. (2004). The reoxidation test was conducted under three different conditions in triplicate well clusters: 1) high nitrate (cluster 1), 2) high nitrate with ethanol (cluster 2), and 3) low nitrate with ethanol (cluster 3) (Table 1). A push-pull test was

Table 1
Summary of biostimulation and reoxidation test methodology.

Test #	Test type	Method	Day(s)	Treatment ID	Well	Amendments
1	Biostimulation	Injection only	0	–	All wells	40 mM EtOH, 20 mM SO_4^{2-}
2	Biostimulation	Injection only	47	–	All wells	40 mM EtOH, 20 mM SO_4^{2-}
3	Biostimulation	Injection only	84	–	All wells	40 mM EtOH, 20 mM SO_4^{2-}
4	Reoxidation	Injection & periodic extraction	139–176	Control	FW224 FW219	30 mM EtOH, 20 mM SO_4^{2-}
4	Reoxidation	Injection & periodic extraction	139–176	Cluster 1	FW220 FW225 FW218	120 mM NO_3^-
4	Reoxidation	Injection & periodic extraction	139–176	Cluster 2	FW226 FW227 FW221	30 mM EtOH, 120 mM NO_3^-
4	Reoxidation	Injection & periodic extraction	139–176	Cluster 3	FW222 FW223	30 mM EtOH, 2 mM NO_3^-

EtOH = ethanol.

Table 2
Pre-test nitrate, U(VI), and sulfate concentrations and pH in source well (GW835) used for test injectate and in wells used for push-pull tests (FW218 through FW227).

Well	NO_3^- (mM)	U(VI) (μM)	SO_4^{2-} (mM)	pH
GW835	1.0	5.0	1.0	6.5
FW218	12.9	0.1	0.4	7.0
FW219	0.4	3.9	0.6	7.4
FW220	0.2	0.1	0.1	7.7
FW221	1.2	0.1	0.2	7.5
FW222	0.1	0.1	0.1	7.8
FW223	0.1	0.1	0.1	8.0
FW224	0.1	0.1	0.1	7.7
FW225	0.7	0.2	0.1	7.5
FW226	1.2	0.2	1.9	7.2
FW227	0.3	0.1	0.5	6.6

conducted in a single well (FW224) under similar ethanol- and sulfate-amended conditions of the biostimulation tests to serve as a control (Table 1). Immediately prior to injection, the injectate was amended with 10 mM sodium bicarbonate ($NaHCO_3$) buffer, 1.3 mM bromide tracer (KBr), and ethanol (C_2H_6O), sulfate (Na_2SO_4) or nitrate (KNO_3), depending on the test condition (Table 1). The reoxidation test injectate volume, mixing and injection methodology, and injection time frame were identical to the biostimulation tests. Five samples of the injectate were collected during the injection phase. Post-injection groundwater samples were collected by periodic extraction of the test wells for 36 days and analyzed for bromide, ethanol, nitrate, nitrite, U(VI), and sulfate.

2.3. Laboratory analysis

Bromide, nitrate, nitrite, and sulfate were measured by ion chromatography (Dionex, model DX-120). U(VI) was measured by a kinetic phosphorescence analyzer (Chemcheck, KPA-11). pH was measured by glass electrode (Accumet, model 25). Ethanol was measured by gas chromatography (Hewlett-Packard, model 5880) with flame ionization detection.

2.4. Data analysis

Dilution-adjusted concentrations were computed by dividing the measured concentration of the reactive tracer (ethanol, nitrate, nitrite, U(VI), and sulfate) by the relative concentration of the non-reactive tracer (bromide) (Istok, 2013). Recovery factors of reactive tracers were computed by dividing the mass extracted from the well by the mass injected into the well which was then divided by the corresponding recovery factor of bromide (Senko et al., 2002). Recovery factors greater than one indicated that more reactive tracer was recovered relative to bromide. Recovery factors less than one indicated that less reactive tracer was recovered relative to bromide.

3. Results and discussion

3.1. Push–pull tests: uranium and sulfate reduction in control well

Complete removal of ethanol occurred within 24 hours after injection and ethanol concentrations remained below the method detection limit for the duration of the 36-day test (Fig. 1). U(VI) concentrations remained below injection levels (5 μM) for the first 13 days of the test (Fig. 1). Complete removal of sulfate occurred within 3 days after injection and sulfate concentrations remained below pre-test levels (0.1 mM) for the first 15 days of the test (Fig. 1). Nitrate and nitrite concentrations and pH remained at pre-test levels for the duration of the 36-day test (data not shown). The observed removal of ethanol and sequential removal of U(VI) and sulfate suggested that microbial-mediated U(VI) and sulfate reduction occurred in the control well for the first 15 days of the test. Although ferrous iron was not measured, it is likely that ferric-iron reduction also occurred based on previous studies in Area 2 where the classic sequence of TEAs were observed in ethanol-amended tests with nitrate reduction, ferric-iron reduction, sulfate reduction, and methanogenesis proceeding in sequence (Mohanty et al., 2008; Fang et al., 2006). These results suggested that groundwater conditions conducive to U(VI) reduction/immobilization and precipitation of reduced sulfur-bearing species were likely established in the first three

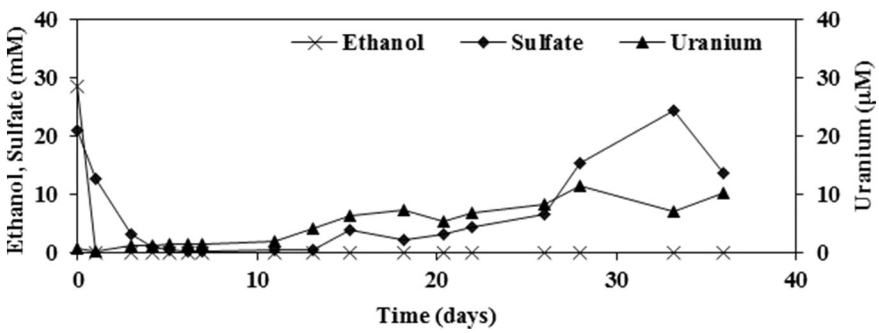

Fig. 1. Dilution-adjusted concentrations of ethanol, sulfate, and U(VI) in control well FW224 amended with 30 mM ethanol and 20 mM sulfate.

biostimulation tests (Table 2). Although the valence state and chemical speciation of uranium and sulfur in sediments were not determined, it is likely that U(VI) was reduced to U(IV) in the form of uraninite and/or as U(IV) adsorbed to Fe/Mn minerals and that sulfate was reduced to S^{2-} in the form of ferrous sulfide (FeS), based on previous ethanol-amended tests at the OR-IFRC site (Kelly et al., 2008, 2010).

Sulfate and U(VI) concentrations increased steadily after 15 days and approached or slightly exceeded injection levels of 20 mM and 5 μM, respectively, by the end of the 36-day test (Fig. 1). The increase of sulfate and U(VI) levels suggested that reoxidation of reduced sulfur-bearing species and U(IV) and/or desorption of sulfate and U(VI) may have occurred. Although sulfate-reducing conditions were clearly established during the first 15 days of the test and nitrate and nitrite concentrations remained at pre-test levels, it is possible that solid-phase oxidants such as Fe(III)-oxides and/or Mn(IV)-oxides were present due to incomplete reduction and were responsible for reoxidation of reduced sulfur-bearing species and U(IV). For example, in a flow-through sediment column study utilizing sediment from Area 2, Wan et al. (2005) provided several lines of evidence which suggested that despite constant electron donor (lactate) addition and strongly methanogenic conditions, Fe(III) and possibly Mn(IV) persisted as oxidants responsible for U(IV) reoxidation. Thermodynamically, any oxidant of U(IV) would be expected to oxidize sulfides preferentially and complete oxidation of FeS and FeS_2 to sulfate by MnO_2 has been observed in marine sediments (Aller and Rude, 1988; Schippers and Jorgensen, 2001). Although there is slight or no evidence for complete FeS or FeS_2 oxidation by Fe(III)-oxides (Aller and Rude, 1988; Schippers and Jorgensen, 2001, 2002), intermediate oxidation products such as elemental sulfur (S^0) and thiosulfate ($S_2O_3^{2-}$) can be completely oxidized to sulfate by microbes which utilize Fe(III)-oxides as TEAs (Finster et al., 1998; Thamdrup et al., 1993). Although desorption of sulfate and/or U(VI) may have also occurred after 15 days it is unlikely due to relatively little change in pH (data not shown) (Barnett et al., 2002; Rose and Ghazi, 1997).

Recovery factors for U(VI) and sulfate were computed in order to quantify the extent of U(VI) and sulfate immobilization/mobilization for duration of the 36-day tests (Table 3). Recovery factors for U(VI) and sulfate were 0.2 and 0.5, respectively, for the control-well test (Table 3). Although both immobilization (0 to 15 days) and mobilization (15 to 36 days) of U(VI) and sulfate were observed (Fig. 1), the recovery factor results suggested that a net removal (recovery factor < 1) of U(VI) and sulfate from groundwater occurred over the full duration of the test (Table 3). Therefore, it is likely that a net removal of U(VI) and sulfate from groundwater by microbial-mediated reduction also occurred during the first three biostimulation tests (Table 1).

3.2. Push–pull tests: uranium mobility in the presence of high nitrate

Complete removal of high nitrate (170 mM) in the absence of ethanol was concurrent with a steady increase in sulfate concentrations above injection levels (up to 25 mM) and a transient increase in nitrite concentrations (up to 2 mM) in well FW220 (Fig. 2). U(VI) concentrations remained near injection levels (5 μM) for the first 28 days of the test and then increased to 20 μM by the end of the 36-day test (Fig. 2). The increase in U(VI) concentrations above injection levels occurred in the absence of detectable nitrate or nitrite (Fig. 2). The pH in well FW220 remained at pre-test levels for the duration of the test (data not shown). Similar results were observed in replicate wells FW219 and FW225 (Supporting Information (SI) Fig. S1) which suggested that despite the high level of aquifer heterogeneity in Area 2 (Watson et al., 2004), the biogeochemical processes were not spatially-biased under test conditions. These results suggested that nitrate reduction was predominantly coupled to reduced sulfur oxidation and that U(IV) oxidation was negligible during this process. These results were expected because preferential oxidation of common reduced sulfur-bearing species such as pyrite, mackinawite, alabandite and elemental sulfur by nitrate or nitrite are thermodynamically favorable when compared to uraninite (Dean, 1999). Although we did not determine the extent at which this process was either abiotic or microbial-mediated, it is important to note that the microbial species *Thiobacillus denitrificans* has been shown to perform nitrate reduction coupled to reduced sulfur oxidation (Kelly and Wood, 2000) and that the *Thiobacillus* genus has been broadly detected at the OR-IFRC site in both groundwater and sediments (Spain and Krumholz, 2011). However, these results also suggested that solid-phase oxidants such as Fe(III)-oxides and/or Mn(IV)-oxides may have been responsible for reoxidation of reduced sulfur-bearing species and U(IV) during the later stages of the tests when nitrate and

Table 3
Recovery factors for U(VI) and sulfate for control (FW224) and test well triplicates during push-pull test 4. Average recovery factors ± one standard deviation are shown for triplicate test wells.

Treatment ID	Well	Amendments	U(VI)	SO_4^{2-}	Avg. U(VI) ± 1 S.D.	Avg. SO_4^{2-} ± 1 S.D.
Control	FW224	30 mM EtOH, 20 mM SO_4^{2-}	0.2	0.5	NA	NA
	FW219		1.0	14.4		
Cluster 1	FW220	120 mM NO_3^-	1.5	8.6	1.3 ± 0.3	12 ± 3
	FW225		1.5	13.0		
	FW218		0.9	9.8		
Cluster 2	FW226	30 mM EtOH, 120 mM NO_3^-	1.8	13.2	1.3 ± 0.4	14 ± 5
	FW227		1.2	20.2		
	FW221		0.5	4.2		
Cluster 3	FW222	30 mM EtOH, 2 mM NO_3^-	1.3	5.6	0.7 ± 0.5	5.5 ± 1.3
	FW223		0.4	6.8		

NA = not applicable, EtOH = ethanol.

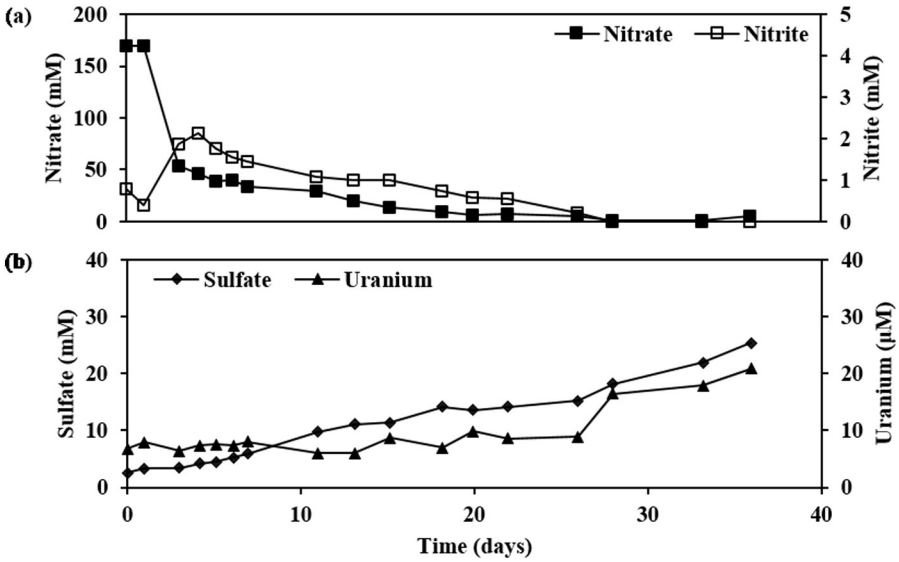

Fig. 2. Dilution-adjusted concentrations of nitrate and nitrite (a) and sulfate and U(VI) (b) in well FW220 amended with 120 mM nitrate.

nitrite concentrations were below the method detection limit (Figs. 2 and S1). Similar results were observed in the control well (Fig. 1) and were discussed in the previous section.

Average recovery factors, plus or minus one standard deviation, for U(VI) and sulfate in the triplicate well cluster 1 were 1.3 ± 0.3 and 12 ± 3, respectively (Table 3). These results demonstrated that substantially more sulfate, but not U(VI), was recovered relative to bromide. The calculated recovery factors for sulfate and U(VI) (Table 3) and the observed nitrate removal and concurrent sulfate production (Figs. 2 and S1) strongly suggested that reoxidation of uranium under nitrate-reducing conditions was substantially limited by preferential oxidation of reduced sulfur-bearing species.

3.3. Push–pull tests: uranium mobility in the presence of high nitrate and ethanol

Removal of high nitrate (140 mM) and ethanol (30 mM) was concurrent with a sharp increase in nitrite concentrations (up to 4 mM) in well FW226 for the first 7 days of the test (Fig. 3). During this time, sulfate concentrations increased steadily (up to 10 mM) while U(VI) concentrations varied but were relatively close to injection levels (5 μM) (Fig. 3).

The results for the first 7 days suggested that nitrate reduction was coupled to both ethanol and sulfur oxidation and that U(IV) oxidation was negligible during this process. Sulfur oxidation by nitrate was expected because nitrate was

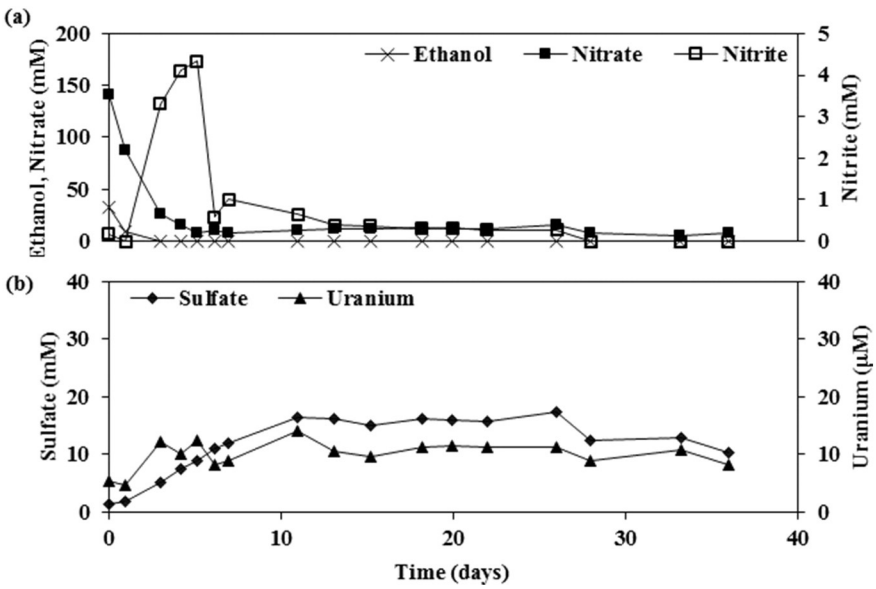

Fig. 3. Dilution-adjusted concentrations of nitrate and nitrite (a) and sulfate and U(VI) (b) in well FW226 amended with 30 mM ethanol and 120 mM nitrate.

added in excess (≈1.5-fold) of the stoichiometric demand for ethanol oxidation (Table 1). After day 7, concentrations of sulfate remained well above injection levels (up to 18 mM) while U(VI) concentrations were only slightly above injection levels (up to 10 μM) until day 28 (Fig. 3). During this time, concentrations of nitrate and nitrite were relatively low but detectable (Fig. 3). The results between days 7 and 28 suggested that a substantial amount of reduced sulfur-bearing species were oxidized to sulfate under nitrate-reducing conditions and that reoxidation of U(IV) was negligible. The concentrations of nitrate and nitrite between days 28 and 36 decreased to below the method detection limit, during which time, concentrations of sulfate and U(VI) also decreased (Fig. 3). These results suggested that oxidation of reduced sulfur-bearing species and U(IV) was nitrate dependent. The pH in well FW226 remained at pre-test levels for the duration of the test (data not shown). Similar results were observed in replicate wells FW218 and FW227 (Fig. S1).

Average recovery factors, plus or minus one standard deviation, for U(VI) and sulfate in the triplicate well cluster 2 were 1.3 ± 0.4 and 14 ± 5, respectively (Table 3). These results demonstrated that substantially more sulfate, but not U(VI), was recovered relative to bromide. However, these results also suggested that adding ethanol had a negligible effect on limiting the oxidation of sulfur and/or U(IV) by high nitrate as made evident by the similar recovery factors for sulfate and U(VI) in the high nitrate (cluster 1) and high nitrate with ethanol (cluster 2) treatments (Table 3). Nevertheless, the calculated recovery factors for sulfate and U(VI) (Table 3) and the observed nitrate removal and concurrent sulfate production (Figs. 3 and S1) strongly suggested that reoxidation of uranium under nitrate-reducing conditions was substantially limited by preferential oxidation of reduced sulfur-bearing species.

3.4. Push–pull tests: uranium mobility in the presence of low nitrate and ethanol

Removal of low nitrate (2 mM) and ethanol (30 mM) was concurrent with a sharp increase in nitrite concentrations (up to 2 mM) in well FW222 for the first 3 days of the test (Fig. 4). During this time, sulfate and U(VI) concentrations increased sharply (up to 30 mM and 30 μM, respectively) (Fig. 4). These results suggested that nitrate reduction was coupled to ethanol, sulfur and U(IV) oxidation. Sulfur and U(IV) oxidation by nitrate was not expected because ethanol was added in excess (40-fold) of the stoichiometric demand for nitrate reduction (Table 1). After day 3, nitrate, nitrite, sulfate and U(VI) concentrations decreased sharply and remained low until day 26 (Fig. 4). After day 26, sulfate concentrations increased sharply (up to 35 mM) in the presence of relatively low nitrate and nitrite while U(VI) concentrations remained near injection levels (5 μM) (Fig. 4).

These results suggested that preferential reoxidation of reduced sulfur-bearing species, as opposed to reoxidation of U(IV), occurred after day 26 in well FW222. However, sulfate and U(VI) concentrations increased to levels which greatly exceeded injection concentrations in the presence of relatively low nitrate and nitrite during later stages of the test in the replicate wells FW221 and FW223 (Fig. S1). These results suggested that concurrent reoxidation of reduced sulfur-bearing species and U(IV) occurred after day 26 wells FW221 and FW223 and indicated that an oxidant in addition to nitrate and nitrite may be have been present. The pH in well cluster 3 remained at pre-test levels for the duration of the tests (data not shown).

Average recovery factors, plus or minus one standard deviation, for U(VI) and sulfate in the triplicate well cluster 3 were 0.7 ± 0.5 and 5.5 ± 1.3, respectively (Table 3). These

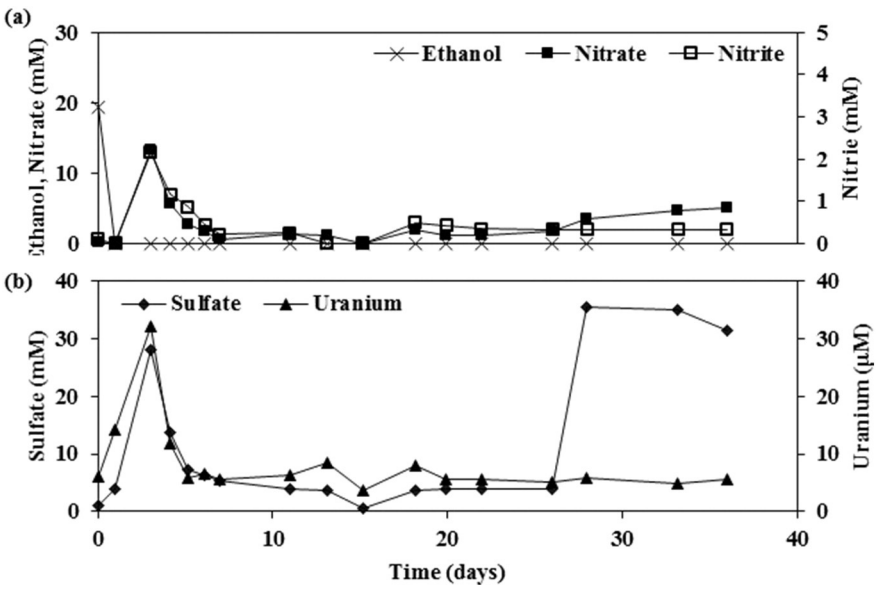

Fig. 4. Dilution-adjusted concentrations of nitrate and nitrite (a) and sulfate and U(VI) (b) in well FW222 amended with 30 mM ethanol and 2 mM nitrate.

Table 4
Standard-state (25 °C, 1 atm, and unit molality) Gibbs free energies of uraninite (UO_2) and various reduced sulfur-bearing species (S^0, FeS, FeS_2, MnS) reoxidized by nitrate (NO_3^-) and nitrite (NO_2^-). Free energy values for the formation of reactants and products were obtained from Dean (1999).

Reaction #	Reaction stoichiometry	ΔG_r^o (kJ)
	Nitrate as oxidant	
1	$UO_2 + 0.4NO_3^- + 2.4H^+ \rightarrow UO_2^{2+} + 0.2N_2 + 1.2H_2O$	−162
2	$S^0 + 1.2NO_3^- + 0.4H_2O \rightarrow SO_4^{2-} + 0.6N_2 + 0.8H^+$	−516
3	$FeS + 1.6NO_3^- + 1.6H^+ \rightarrow SO_4^{2-} + 0.8N_2 + Fe^{2+} + 0.8H_2O$	−735
4	$FeS_2 + 2.8NO_3^- + 0.8H^+ \rightarrow 2SO_4^{2-} + 1.4N_2 + Fe^{2+} + 0.4H_2O$	−1184
5	$MnS + 1.6NO_3^- + 1.6H^+ \rightarrow SO_4^{2-} + 0.8N_2 + Mn^{2+} + 0.8H_2O$	−766
	Nitrite as oxidant	
6	$UO_2 + 0.7NO_2^- + 2.7H^+ \rightarrow UO_2^{2+} + 0.3N_2 + 1.3H_2O$	−216
7	$S^0 + 2NO_2^- \rightarrow SO_4^{2-} + N_2$	−680
8	$FeS + 2.7NO_2^- + 2.8H^+ \rightarrow SO_4^{2-} + 1.35N_2 + Fe^{2+} + 1.4H_2O$	−968
9	$FeS_2 + 4.7NO_2^- + 2.8H^+ \rightarrow 2SO_4^{2-}2.35N_2 + Fe^{2+} + 1.4H_2O$	−1582
10	$MnS + 2.7NO_2^- + 2.8H^+ \rightarrow SO_4^{2-} + 1.35N_2 + Mn^{2+} + 1.4H_2O$	−999

results demonstrated that substantially more sulfate, but not U(VI), was recovered relative to bromide. These results also suggested that low nitrate had a noticeable effect on limiting the oxidation of sulfur and/or U(IV) as evident by the higher recovery factors for sulfate and U(VI) in the high nitrate (cluster 1) and high nitrate with ethanol (cluster 2) treatments (Table 3).

3.5. Thermodynamics

The standard-state Gibbs free energies of several simple redox reactions that may have occurred during the reoxidation tests were computed (Table 4) in order to compare to the experimental data from the reoxidation tests. It is important to recognize that standard-state conditions (25 °C, 1 atm, and unit molality) may yield Gibbs free energies that are different than those calculated under system-specific conditions. The energetics of nitrate oxidation of reduced sulfur-bearing species that were likely formed during the biostimulation tests (S^0, FeS, FeS_2, MnS,) were substantially more favorable than for the oxidation of uraninite (Table 4). Similar energetics were calculated for nitrite as the oxidant (Table 4). The energetics of the predicted reoxidation reactions were comparable to the computed recovery factors for sulfate and U(VI) under nitrate-reducing conditions as evident by substantially more sulfate recovered when compared to U(VI) during all three reoxidation tests (Table 3). This comparison further suggested that preferential oxidation of reduced sulfur-bearing species by nitrate and/or nitrite, as predicted thermodynamically, was also observed in this study under in situ conditions. However, the in situ data also suggested that concurrent reoxidation of reduced sulfur-bearing species and U(IV) did occur under both nitrate-reducing conditions and conditions in which nitrate and/or nitrite concentrations were not detectable (Figs. 2, 3, and 4); although to a much lesser extent for U(IV) (Table 3), which does not fully agree with the energetics (Table 4). This suggested that the system-specific conditions may yield different energetics and/or that we did not identify all of the predominant redox reactions (Table 4).

4. Conclusions

The results of this study suggested that the in situ mobility of uranium under nitrate-reducing conditions can be substantially limited by preferential oxidation of reduced sulfur-bearing species. This study also suggested that the addition of ethanol can result in less reoxidation of uranium by nitrate if added in substantial excess of the stoichiometric demand of nitrate as an electron acceptor. The thermodynamics of the predicted reoxidation reactions were supported by the in situ data and suggested that thermodynamically-favorable oxidation of common reduced sulfur-bearing minerals by nitrate and/or nitrite, as opposed to oxidation of uraninite, likely occurred. However, concurrent oxidation of reduced sulfur-bearing species and to a much lesser extent, U(IV), was also observed under nitrate-reducing conditions and in the absence of detectable nitrate and/or nitrite. This suggested that reduced sulfur-bearing species were not fully effective at limiting the mobility of uranium in the presence of dissolved and/or solid-phase oxidants. Therefore, future in situ studies designed to test the effectiveness and long-term sustainability of this approach under natural-gradient conditions and to elucidate the predominant redox reactions are needed. Nevertheless, this in situ study confirmed the results of previous laboratory studies and demonstrated that establishing sulfate-reducing conditions following U(VI) reduction can substantially limit the extent of uranium mobility in the presence of nitrate oxidant.

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