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Biological reduction of uranium in groundwater and subsurface soil

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Abstract

Biological reduction of uranium is one of the techniques currently studied for in situ remediation of groundwater and subsurface soil. We investigated U(VI) reduction in groundwaters and soils of different origin to verify the presence of bacteria capable of U(VI) reduction. The groundwaters originated from mill tailings sites with U concentrations as high as 50 mg/l, and from other sites where uranium is not a contaminant, but was added in the laboratory to reach concentrations up to 11 mg/l. All waters contained nitrate and sulfate. After oxygen and nitrate reduction, U(VI) was reduced by sulfate-reducing bacteria, whose growth was stimulated by ethanol and tri*meta*phosphate. Uranium precipitated as hydrated uraninite ($UO_2 \cdot xH_2O$). In the course of reduction of U(VI), Mn(IV) and Fe(III) from the soil were reduced as well. During uraninite precipitation a comparatively large mass of iron sulfides formed and served as a redox buffer. If the excess of iron sulfide is large enough, uraninite will not be oxidized by oxygenated groundwater. We show that bacteria capable of reducing U(VI) to U(IV) are ubiquitous in nature. The uranium reducers are primarily sulfate reducers and are stimulated by adding nutrients to the groundwater.

Keywords: Uranium; Bioremediation; Groundwater; Uraninite; Iron sulfide; Indigenous bacteria; Speciation; Redox buffer

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1. Introduction

Biological reduction of uranium has been proposed as a technique for uranium removal from groundwaters via reductive precipitation (Kauffman et al., 1986; Francis et al., 1991, 1994; Lovley et al., 1991, 1993; Gorby and Lovley, 1992; Lovley and Phillips, 1992a,b; Barnes and Cochran, 1993; Lovley, 1995; Phillips et al., 1995; Barton et al., 1996; Uhrie et al., 1996; Tucker et al., 1996, 1998a,b; Hard et al., 1997; Ganesh et al., 1997; Abdelouas et al., 1998a, 1999a,b). These authors showed that aqueous uranium can be reduced by a variety of microorganisms including iron- and sulfate-reducing bacteria and in some cases by denitrifying bacteria. The product of uranium reduction is uraninite, UO_2, a highly insoluble mineral under reducing conditions (Langmuir, 1978; Parks and Pohl, 1988). In nature, reduction of U(VI) in anoxic marine sediments is the most important sink of dissolved uranium (e.g. Cochran et al., 1986; Klinkhammer and Palmer, 1991). Reduction of U(VI) in the subsurface environment lead to the formation of uranium ore deposits (Jensen, 1958; Hosteler and Garrels, 1962; Taylor, 1979; Maynard, 1983). Uraninite and pitchblende, both nominally UO_2, are the principal ore minerals in many ore deposits (Rich et al., 1977; Kimberley, 1979). Natural uraninite is fairly stable over geological time. For instance, 2 billion-year-old uranium ore deposits are known in Oklo (Gabon) (Gauthier-Lafaye and Weber, 1989; Gauthier-Lafaye et al., 1989, 1996, 1997; Nagy et al., 1991; Bros et al., 1993). The stability of uraninite at the Oklo deposits was sustained by the presence of siderite ($FeCO_3$), pyrite (FeS_2) and organic matter in the form of bitumen, which consumed the oxygen supplied by infiltrating groundwater (Blanc, 1995; Janeczek, 1999). Abdelouas et al. (1999a) reported that oxidation of biologically reduced uranium increased with increasing ratio of dissolved oxygen/uraninite. In the present work we study the effect of iron sulfide/uraninite ratio on U(IV) oxidation.

A recent study (Quinton et al., 1997) showed that among the groundwater cleanup technologies — pump and treat, permeable reactive barrier with zero-valent iron granular filings, and a biobarrier, intrinsic or engineered in situ bioremediation — the latter is the most cost-effective. In situ bioremediation consists of the activation of indigenous microbial populations to degrade or precipitate the contaminants (National Research Council, 1994). A conventional technique such as 'pump and treat' may not be adequate for uranium removal because pumping the water may change the uranium speciation followed by sorption of uranium on the host rock (Abdelouas et al., 1998b). With in situ bioremediation both soluble and sorbed U(VI) can be reduced and immobilized by bacteria. To date in situ biological remediation of uranium has not been demonstrated in the field. In natural aquifers mixed cultures of nitrate-, metal- and sulfate-reducing bacteria are likely to be present (Hodgkinson, 1987; Ghiorse, 1997; Nealson and Stahl, 1997; Bachofen et al., 1998). In the presence of carbon, nitrogen and phosphorus sources and adequate respective electron acceptors, these bacteria will be stimulated in the following order: denitrifying bacteria, metal-reducing bacteria, and finally sulfate-reducing bacteria (Nealson and Stahl, 1997; Lu, 1998; Abdelouas et al., 1998a).

Several laboratory studies have been devoted to the enzymatic reduction of uranium under a variety of conditions relevant to ex situ treatments of waste streams from radionuclide processing facilities (e.g. Macaskie, 1991; Ganesh et al., 1997). These studies used pure strains of bacteria (e.g. desulfovibrio species) to elucidate the impact of inorganic (e.g. nitrate, sulfate, bicarbonate) and organic (e.g. acetate, malonate, oxalate, citrate) ions on uranium removal from waste waters. Only a few studies focused on uranium reduction with mixed cultures of bacteria in groundwaters (Barton et al., 1996; Ganesh et al., 1997; Abdelouas et al., 1998a). In the case of in situ bioremediation the presence of mixed-culture of bacteria is a prerequisite for uranium reduction.

The objective of this study is to determine whether bacteria capable of uranium reduction are encountered in groundwaters and soils from different locations, and whether they can be easily activated.

2. Experimental

2.1. Groundwater and soil

Groundwaters and soils were collected in autoclaved 1-l plastic containers and in 160-ml serum bottles placed in a nitrogen flushed glove box in the field. Temperature, pH, dissolved oxygen were measured either in situ using a YSI-6920 probe (YSI, OH, USA) or using samples in the glove box after the well had been pumped extensively. The bottles with the groundwater and soil were kept under argon atmosphere to avoid oxidation of samples in a refrigerator at 4°C without additives. Water and soil samples were used within the first week following their collection to conduct experiments of biological reduction of uranium. In the past we found that long storage of groundwater resulted in a significant decrease of the number of viable bacteria including denitrifying and sulfate-reducing bacteria (Lu, 1998). Furthermore, prolonged storage of groundwater can also affect its geochemistry such as calcium carbonate precipitation and change in pH (Abdelouas et al., 1998b).

Groundwater compositions are given in Table 1. One groundwater sample (well #926) originated from the mill tailings site near Tuba City, AZ (USA), four groundwater samples (GW1–GW4) came from mining and tailing site in Germany, two groundwater samples (NMW1 and NMW2) from the mill tailings site in Grants, NM (USA), one groundwater sample from a dairy site in Bernalillo, NM (USA), and one groundwater from a former farm site in Albuquerque, NM (USA). Uranium(VI) concentrations ranged between 0.25 and 50 mg/l, sulfate concentrations between 0.105 and 17.9 g/l, and nitrate concentrations between 0.0085 and 1.2 g/l. All groundwaters showed a pH near neutral except those collected from the mill tailings site near Grants, NM (pH = 10). In this water the alkaline leaching process used to extract uranium from the rock lead to strong enrichment of the groundwater with carbonate (1.3×10^{-1} M), which may inhibit uranium biological reduction (Phillips et al., 1995).

2.2. Groundwater amendment

Addition of amendment to the system groundwater/soil was required to activate indigenous bacteria. In the experiments where only organic carbon or phosphorus sources were added to the groundwater and soil, uranium was not reduced. This observation suggested that neither carbon nor phosphorus in groundwater and soil were available to the indigenous bacteria. As a result groundwater amendment with organic carbon and

Table 1
Chemical composition of unamended groundwaters from various locations (mg/l)

	Location of uranium mill tailings sites							Dairy site Bernalillo NM (USA) –	Farm site Albuquerque NM (USA) –
	Tuba City AZ (USA) Well #926	Germany				Grants NM (USA)			
		GW1	GW2	GW3	GW4	NMW1	NMW2		
U(VI)	0.25	0.9	0.77	1.76	3.60	50.0	50.0	3.7[a]	1–11[a]
SO_4^{2-}	1830	457	6300	17 952	14 942	11 353	12 421	234	105
Total Fe	0.05	3.5	2.0	< 0.5	2.0	0.6	1.3	< 0.05	0.03
Total Mn	0.02	0.4	0.06	2.6	0.15	0.1	0.1	0.04	0.05
NO_3^-	1220	52.8	29.0	134	125	8.5	33.5	240	450
Dissolved oxygen	3.1	6.1	6.2	6.2	6.3	6.5	6.4	5.7	4.8
pH	6.6	7.6	7.6	7.7	7.8	10.0	9.9	6.8	7.3
Water level (feet)	40	7	7	7	7	100	100	70	16

[a] Uranium was added to the groundwater in the laboratory.

phosphorus sources was required to stimulate bacterial growth. In previous work (Abdelouas et al., 1998a; Lu, 1998; Lu et al., 1999), the authors tested enzymatic uranium reduction in groundwater using several organic carbons (acetate, methanol, glucose, lactate, ethanol) and phosphorus (*ortho*- and *meta*phosphate) sources and found that ethanol (C_2H_5OH) and sodium tri*meta*phosphate (TMP), $Na_3P_3O_9$, yielded the highest rates of growth of bacteria and uranium reduction. Benner et al. (1997) showed that ethanol is a suitable carbon source for the growth of a mixed-culture of sulfate reducing bacteria to remove zinc from groundwater in an ex situ treatment plant. In the present work, we used ethanol and TMP to amend the groundwater. No pH-buffers or reducing agents were added. The groundwater was amended with the minimum amount of chemicals necessary. The less chemicals added to the groundwater, the lower the overall costs of the remediation and the better the quality of the groundwater at the end of the process.

For denitrification, an ethanol/nitrate ratio was established slightly higher than the stoichiometric one of 5:12 [Eq. (1)].

$$12NO_3^- + 5C_2H_5OH + 2H^+ = 6N_2 + 10HCO_3^- + 11H_2O \quad (1)$$

For uranium and sulfate reduction, the ethanol/sulfate ratio was 2:3 [Eq. (2)] for the groundwaters with low sulfate concentration.

$$3SO_4^{2-} + 2C_2H_5OH = 4HCO_3^- + 3HS^- + H^+ + 2H_2O \quad (2)$$

To the groundwaters with high sulfate concentrations just enough ethanol was added to reduce 3–5 mM SO_4^{2-} together with uranium, which resulted in addition of 2–3.3 mM of ethanol to 100 ml of groundwater. During the reduction of 3–5 mM SO_4^{2-} in groundwater, U(VI) at a concentration of 1–10 mg/l was entirely reduced. Furthermore, for water with high uranium concentration (mill tailings site in Grants) more ethanol was added according to Eq. (3).

$$6UO_2(CO_3)_2^{2-} + C_2H_5OH + 5H_2O = 6UO_2 + 14HCO_3^- + 2H^+ \quad (3)$$

TMP was added to the groundwater to reach a final concentration of PO_4^{2-} of 20 mg/l, which yielded the highest rate of sulfate and uranium reduction. Eqs. (1)–(3) neglect biomass formation, but a small fraction of the carbon will be incorporated into bacterial biosynthesis.

2.3. Batch experiments

Stock solutions of 0.5 M ethanol and 7×10^{-2} M of TMP were prepared and transferred into serum bottles. The bottles were then purged with argon to remove oxygen and autoclaved at 120°C for 25 min.

The experiments were conducted in serum bottles shortly after sample collection. For each experiment 100 ml of groundwater and 8 g of soil were used. The bottles were sealed with a butyl rubber stopper in an aseptic environment in a glove box, crimped with an aluminum seal, and were removed from the glove box. A syringe needle was introduced through the stopper to purge the groundwater with argon to establish an anaerobic environment. The reaction progress was monitored by collecting aliquots of 2 ml using a sterile 3-ml syringe for chemical analysis. The reaction progress was indicated by precipitation of black compounds, presumably iron sulfides and uraninite. At the end of the reaction the final volume of water was between 80 and 90 ml. Control experiments were conducted to distinguish between biotic and abiotic reduction of U(VI). In these experiments the microorganisms were killed by heat before addition of amendments.

Groundwater with low sulfate concentration was doped with sulfate $FeSO_4 \cdot 7H_2O$ or Na_2SO_4 (1 g/l sulfate) to determine the impact of sulfate concentration on uranium reduction and dissolution/oxidation, and to obtain enough iron sulfide for identification. Precipitation of iron sulfide can help protect uraninite from dissolution/oxidation by flowing oxygenated groundwater following in situ bioremediation. To groundwater from the

Tuba City mill tailings site uranyl nitrate $[UO_2(NO_3)_2 \cdot 6H_2O]$, was added to obtain enough uraninite for identification.

In some experiments sulfate-reducing bacteria were cultivated in batch experiments using untreated groundwater/soil by adding ethanol and TMP. The growth of sulfate-reducing bacteria was indicated by the reduction of sulfate and formation of H_2S and iron sulfide. Aliquots of the cultures (5–10 ml) were added to some experiments to enhance reduction of uranium.

In the experiments with variable molar ratio of uraninite/iron sulfide, the bioremediated water was replaced by uncontaminated naturally oxygenated groundwater from the Tuba City site. The reoxidation of uraninite and iron sulfide was determined by measuring U(VI) and sulfate in solution.

2.4. Analytical procedures

Prior to analysis, groundwaters were passed

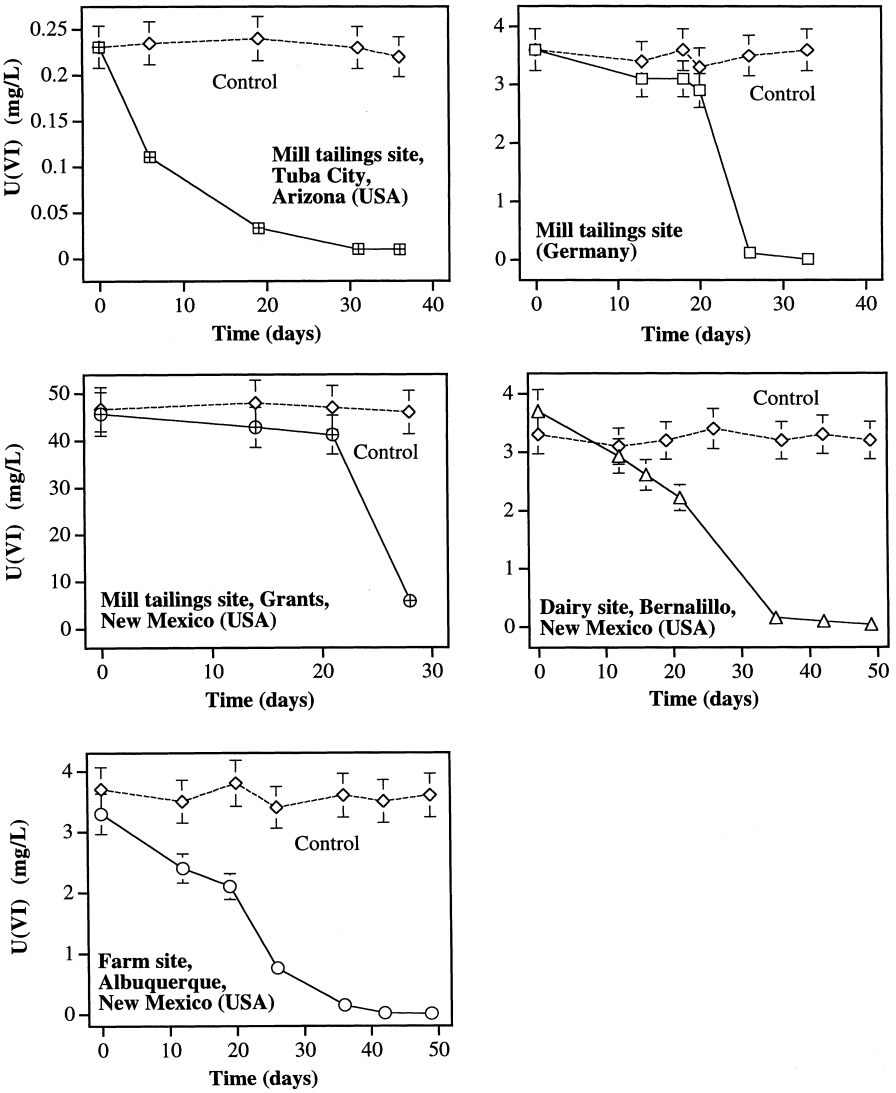

Fig. 1. Reduction of uranium in groundwaters amended with ethanol and tri*meta*phosphate at 24°C.

through a nylon Acrodisc syringe filter with a 0.2-μm pore size to remove biomass and mineral particles from the soil. Uranium was analyzed using a laser fluorescence analyzer (Scintrex UA-3) with a detection limit of 0.5 μg/l and a precision of ±15%. The uranium analyzer detects only hexavalent uranium. Nitrate and sulfate were measured by ion chromatography using a Dionex (DX-500) ion chromatograph with a precision of ±5%. Iron and manganese were measured by atomic absorption spectroscopy with a precision of ±5%. The solid phases containing reduced uranium and iron were identified using a Jeol JEM-2000 FX transmission electron microscope. Ethanol content was not measured.

3. Results and discussions

3.1. Uranium reduction in groundwater and soil

The activity of indigenous bacteria was observed by the production of gas, which increased the pressure in the serum bottles, and by the formation of dark precipitates, presumably iron sulfide and uraninite. The results of uranium reduction in groundwaters are plotted in Fig. 1. In all but the experiments with the groundwater from the mill tailings site (Grants, NM), uranium concentration decreased to a level below the United States groundwater protection standard (44 μg/l) (Federal Register, 1995). At 24°C, the uranium reduction was complete typically within 5 weeks (Fig. 1). In the experiments using the groundwater from the mill tailings at Grants, the uranium concentration decreased by 90% within 4 weeks to reach a final concentration of 5 mg/l. Control experiments with autoclaved groundwater and soil did not show any uranium reduction, suggesting that the reduction of uranium is microbially-mediated. Reduction of uranium by sulfide is possible, but this process is relatively slow. In fact, Abdelouas et al. (1998a) showed that the presence of carbonate and bicarbonate in groundwater inhibits uranium reduction by sulfide. Carbonate and bi-carbonate are common anions in groundwaters (Langmuir, 1997), and are produced by oxidation of organic carbon by bacteria [Eqs. (1)–(3)].

The chemical composition of groundwater at the end of uranium reduction is given in Table 2. Despite the production of H^+ during the reduction of sulfate and uranium, there was no significant change in pH, which underlines the strong buffering capacity of the soil (e.g. Read et al., 1993). Most of the sulfate was reduced to sulfide (S^{2-}) in groundwater with low initial sulfate concentration (GW1, dairy site, farm site). The experiments with high initial sulfate concentration (Tuba city, GW2, GW3, GW4, NMW1, NMW2)

Table 2
Chemical composition of bioremediated groundwaters from various locations (mg/l)

	Location of uranium mill tailings sites							Dairy site Bernalillo NM (USA) –	Farm site Albuquerque NM (USA) –
	Tuba City AZ (USA) Well #926	Germany				Grants NM (USA)			
		GW1	GW2	GW3	GW4	NMW1	NMW2		
U(VI)	0.014	0.004	0.001	0.001	0.001	5.0[a]	4.5[a]	0.001	0.002
SO_4^{2-}	1250	7.7	3657	16 409	10 709	8770	8000	0.5	1.6
Total Fe	0.59	12.6	5.0	1.0	12.6	2.2	18.1	3.5	4.4
Total Mn	0.76	18	22.0	48.0	6.4	0.3	0.1	2.2	1.2
NO_3^-	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Dissolved oxygen	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
pH	6.8	7.3	7.5	7.9	7.6	9.8	9.7	6.7	7.2

[a] The high carbonate concentrations in these solutions (1.3×10^{-1} M) lead to formation of U(VI)-carbonato complexes stable under reducing conditions (Brookins, 1988), which inhibited the complete reduction of U(VI).

showed only partial reduction of sulfate. The reduction of sulfate confirms the activity of sulfate-reducing bacteria. A fraction of Fe(III) and Mn(IV) from the soil was reduced to Fe(II) and Mn(II), respectively. Iron(II) and Mn(II) in solution can be oxidized by dissolved oxygen and precipitated as oxyhydroxides. These are not considered a health hazard (Seelig et al., 1992).

We conducted thermodynamic calculations using the EQ3NR code (Wolery, 1992) to determine the uranium speciation in groundwater and to identify the mineral phases likely to precipitate. As input data, the chemical composition of the waters measured at the end of uranium reduction was used (Table 2). The carbonate concentration was derived from Eqs. (1) and (2). Two E_H values were used, $E_H = -100$ and -300 mV, which are reached at the end of denitrification (Abdelouas et al., 1998a) and under sulfate reducing conditions (Odom and Singleton, 1993), respectively. Hydrogen sulfide concentration was estimated as the difference between the final and initial sulfate concentrations. The results of uranium speciation calculations and saturation index calculations (log Q/K; Q = ion activity product, K = equilibrium constant) of selected minerals are given in Tables 3 and 4, respectively. At near neutral pH, an $E_H = -100$ mV, and relatively low bicarbonate concentration (< 0.05 mM HCO_3^-), uranium speciation is dominated by the species $U(OH)_4(aq)$ and some U(VI)-carbonato complexes (Table 3). The groundwaters are saturated with respect to uraninite and iron sulfides such as pyrite (FeS_2) and pyrrhotite ($Fe_{1-x}S$) (Table 4). Experimentally, mackinawite ($FeS_{0.9}$) and some pyrite and pyrrhotite were identified as the main iron sulfide compounds. Mackinawite does not exist in the EQ3NR code's data base. Mackinawite is a *meta*stable phase and will ultimately be converted to the more stable pyrite (Pósfai et al., 1998). For an E_H of -300 mV, the only uranium species present in solution is $U(OH)_4(aq)$ and uraninite and iron sulfide saturation indices increased, making these phases likely to precipitate. For groundwater from the mill tailings site at Grants, uranium is complexed with carbonate even at $E_H = -300$ mV (Table 3). For an $E_H = -100$ mV, the solution is highly undersaturated with respect to uraninite (log $Q/K = -6.6$), but saturated with respect pyrite and rhodochrosite ($MnCO_3$) (Table 4). Precipitation of rhodochrosite in groundwater from the mill tailings site at Grants, but not in the rest of groundwaters, is possible because of the high pH and

Table 3
Calculated uranium speciation in groundwaters at 24°C[a]

	$E_H = -100$ mV	$E_H = -300$ mV
Mill tailings, Tuba City, AZ (USA), groundwater well #926	73% $U(OH)_4(aq)$ 21% $UO_2(CO_3)_3^{4-}$ 6% $UO_2(CO_3)^{2-}$	100% $U(OH)_4(aq)$
Mill tailings, Germany, groundwater (GW1)	81% $U(OH)_4(aq)$ 12% $UO_2(CO_3)_3^{4-}$ 7% $UO_2(CO_3)^{2-}$	100% $U(OH)_4(aq)$
Mill tailings, Grants, NM (USA), groundwater (NMW1)	100% $UO_2(CO_3)_3^{4-}$	100% $UO_2(CO_3)_3^{4-}$
Dairy site, Bernalillo, NM (USA)	100% $U(OH)_4(aq)$	100% $U(OH)_4(aq)$
Farm site, Albuquerque, NM (USA)	65% $U(OH)_4(aq)$ 40% $UO_2(CO_3)_3^{4-}$ 2% $UO_2(CO_3)^{2-}$	100% $U(OH)_4(aq)$

[a] The composition of the water used in calculations with EQ3NR code is that measured at the end of uranium reduction (Table 2).

Table 4
Saturation indices (log Q/K) of groundwaters at 24°C for U(IV) and Mn(II) phases and iron sulfides[a]

	Uraninite	$UO_{2.25}$	Pyrite (FeS_2)	Pyrrhotite ($Fe_{1-x}S$)	Rhodochrosite ($MnCO_3$)
Mill tailings, Tuba City, AZ (USA), groundwater well #926	+4.9	+2.0	+16.3	+2.9	< −10
Mill tailings, Germany, groundwater (GW1)	+5.3	+3.9	+17.9	+2.4	−4.6
Mill tailings, Grants, NM (USA) groundwater (NMW1)	−6.6 +2.6[b]	−6.3 +0.6[b]	+14.7 +20.3[b]	−0.9 +6.8[b]	+0.9 +0.9[b]
Dairy site, Bernalillo, NM (USA)	+6.2	+4.4	+18.1	+2.0	−1.3
Farm site, Albuquerque, NM (USA)	+5.9	+4.5	+18.1	+2.1	−0.4

[a]The composition of the water used in calculations with EQ3NR code is that measured at the end of uranium reduction (Table 2): $E_H = -100$ mV.
[b]$E_H = -300$ mV.

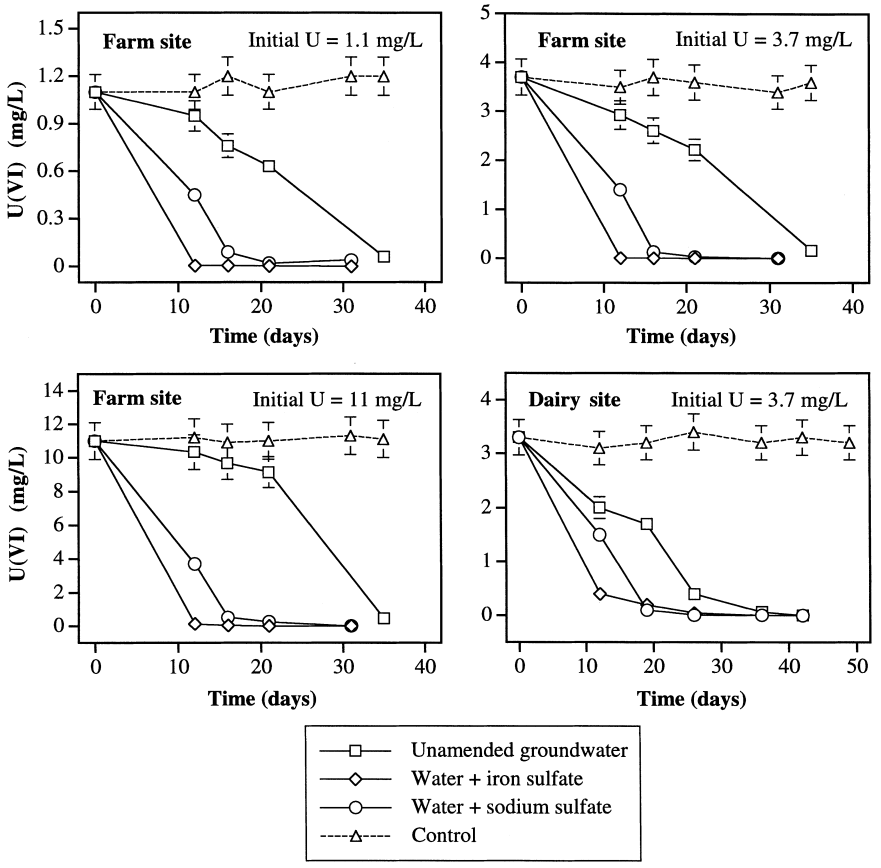

Fig. 2. Effect of sulfate addition on uranium reduction in groundwaters. Iron or sodium sulfate were added to reach a final sulfate concentration of approximately 1 g/l. The initial sulfate concentration in the farm and dairy site groundwaters are 105 and 234 mg/l, respectively.

carbonate concentration in this water (Table 1). However, for an $E_H = -300$ mV the solution is highly super-saturated with respect to uraninite ($\log Q/K = +2.6$). The competition between uranium complexation and reduction is the most likely cause of incomplete reduction of uranium. This result is in agreement with findings by Phillips et al. (1995) who showed that a carbonate concentration of 100 mM inhibited the enzymatic reduction of uranium, while a carbonate concentration of 33 mM had no effect. The presence of sulfide prevents formation of siderite, $FeCO_3$.

3.2. Effect of sulfate concentration on uranium reduction in groundwater / soil

To test the effect of sulfate concentration on U(VI) reduction, sodium or iron sulfate were added to the groundwaters with low sulfate concentrations. After addition of iron sulfate to the water, a yellowish precipitate of Fe(III) hydroxide formed. Results of uranium reduction with/without addition of sulfate are given in Fig. 2. Control experiments using autoclaved groundwater and soil show no reduction of uranium. Reduction of uranium took longer in groundwaters with low sulfate concentration and uranium concentrations between 1.1 and 11 mg/l. Uranium reduction was complete within 5 weeks. Experiments with high sulfate concentration took 12 days (farm site, water + iron sulfate) to 21 days (farm and dairy sites, water + sodium sulfate) to completely reduce uranium. The abundance of sulfate in solution as an electron acceptor for sulfate-reducing bacteria stimulated the growth of these bacteria and enhanced uranium reduction. Uranium(VI) was removed faster in the experiment with iron sulfate than with sodium sulfate probably because of its partial sorption onto the newly formed Fe(III) hydroxides. At the end of the experiment, all the U(VI) sorbed was reduced because all the Fe(III) hydroxide was reduced to form Fe(II) sulfides.

In some experiments iron sulfate was added to reach sulfate concentrations of 0.9, 0.7, 0.5, and 0.3 g/l to determine the concentration of sulfate necessary to yield a high reduction rate of uranium. The results are plotted in Fig. 3. The uranium reduction is slower in water containing 0.5 and 0.3 g/l than in water with sulfate concentrations of 0.7 and 0.9 g/l. In the experiments with low sulfate concentration (≤ 0.5 g/l) uranium was totally reduced within 36 days, while in water with sulfate concentration ≥ 0.7 g/l uranium was reduced within 21 days. Comparing the results in Fig. 3 with those in Fig. 2, we can say that the increase in sulfate concentration in groundwater (farm site) from the initial concentration of 105 mg/l to 0.5 g/l did not affect the reduction rate of uranium. In these experiments, uranium was reduced roughly within 5 weeks. However, for a sulfate concentration ≥ 0.5 g/l uranium reduction was fast. Finally, it took only 12 days to reduce uranium in water completely with 1.1 g/l sulfate.

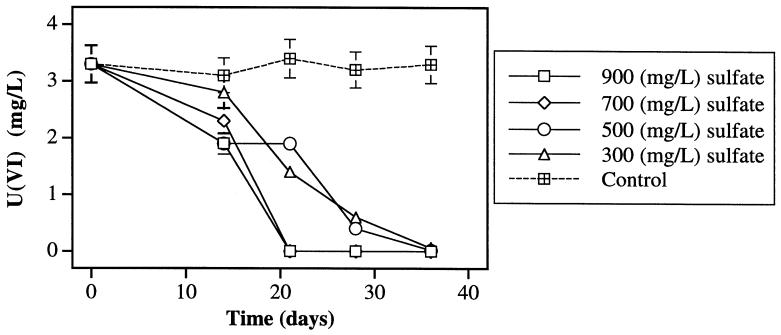

Fig. 3. Effect of sulfate concentration on uranium reduction in groundwater from the farm site.

3.3. Effect of soil treatment on uranium reduction in groundwater / soil

We conducted experiments with groundwater and untreated soil (contains viable bacteria) or autoclaved soil (does not contain viable bacteria). The water and soil samples originated from the mill tailings site in Germany. The results are given in Fig. 4. Control experiments using autoclaved groundwater and soil show no reduction of uranium. Fig. 4 shows that regardless of the composition of the water, uranium was reduced within 13 days in the experiment with untreated soil and groundwater, while it took almost 5 weeks to reduce uranium completely in the experiments with autoclaved soil samples but with untreated water. Inoculation of the samples containing autoclaved soil and untreated water (Fig. 4, square) with cultivated bacteria from the experiments with untreated soil and groundwater (Fig. 4, circle) at day 13 increased the rate of reduction of uranium as can be seen in Fig. 4 (diamond). This result shows that mixed-culture containing indigenous sulfate-reducing bacteria can be grown in batch experiments using groundwater/soil from the contaminated site and can be used to promote uranium reduction, if necessary. In the experiments with untreated soil (Fig. 4, circle), the abundance of viable bacteria in the soil led to a rapid growth of sulfate-reducing bacteria that reduced uranium. In the experiments with autoclaved soil, only the groundwater contained bacteria, resulting in smaller initial populations of bacteria. These results suggest that in situ reduc-

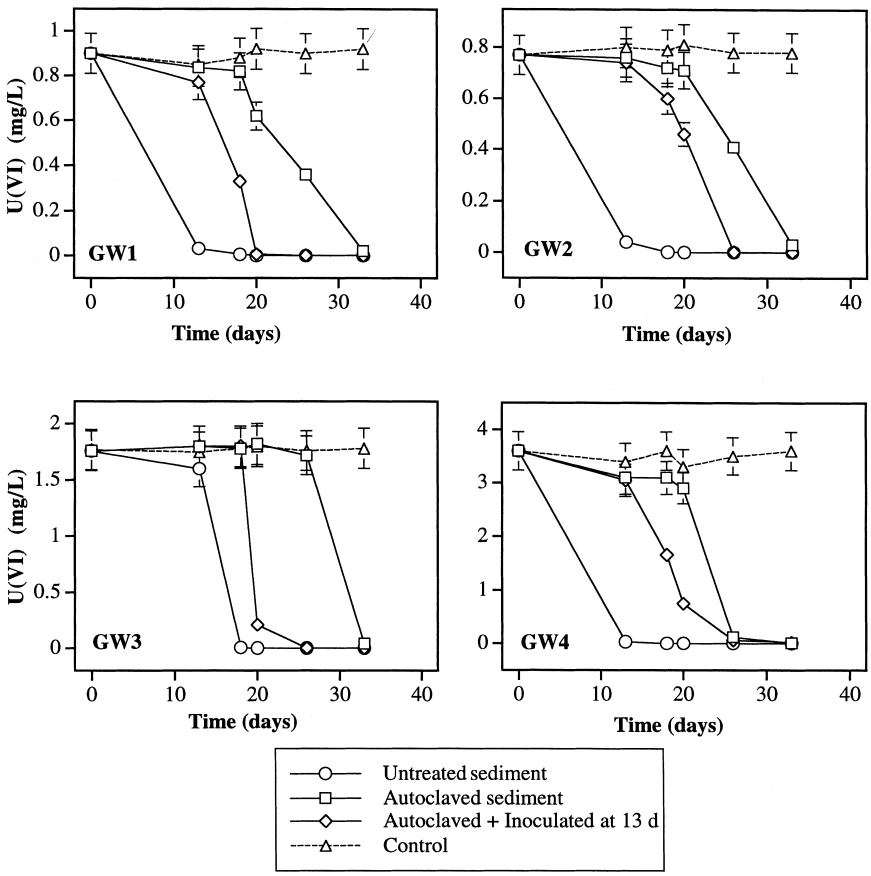

Fig. 4. Effect of soil treatment on uranium reduction in groundwaters from the mill tailings site in Germany.

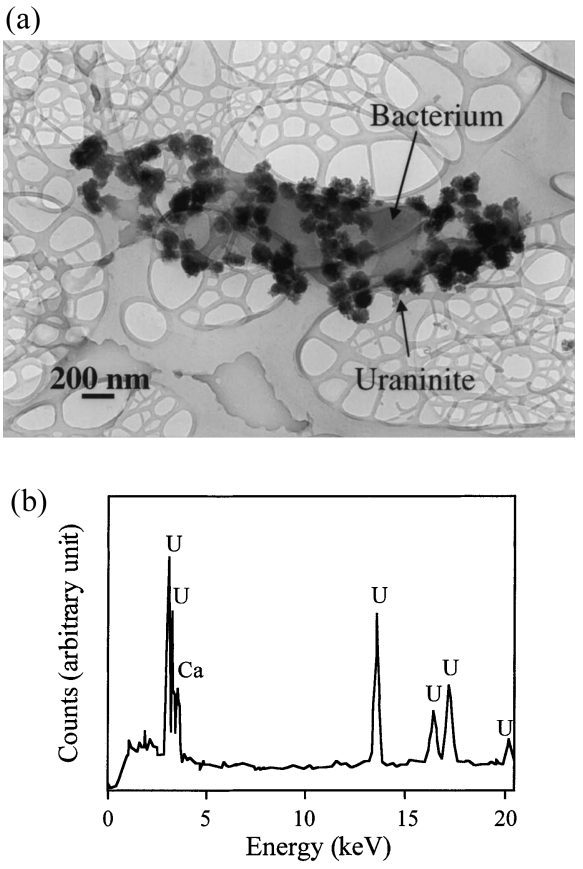

Fig. 5. (a) Bacterium with uraninite particles (mill tailings site, Tuba city); and (b) chemical microanalysis spectrum of uraninite.

tion of uranium by sulfate-reducing bacteria is likely to be faster than in the laboratory because of the high ratio soil/water, providing a high initial concentration of bacteria. In a previous study on in situ biological denitrification, it was found that the in situ reduction of nitrate in groundwater/soil was fast and complete within 5 days, while it took up to 15 days in the laboratory using batch experiments (Deng, 1998; Abdelouas et al., 1999c).

3.4. Importance of iron sulfide formation during in situ bioremediation of uranium

An example of uraninite that precipitated from the Tuba city groundwater after enzymatic reduction of uranium is given in Fig. 5a. Uraninite particles are attached to a bacterium. An example of chemical microanalysis spectrum of a uraninite particle is shown in Fig. 5b. Mackinawite, and some pyrite and pyrrhotite were encountered in the experiments. Fig. 6a,b and Fig. 7a,b show mackinawite and pyrite, formed by reduction of Fe(III) to Fe(II) and SO_4^{2-} to S^0 and S^{2-}, and their chemical microanalysis spectra. The results of the thermodynamic calculations in Table 4 are in agreement with the experimental findings.

It is important to consider uraninite reoxidation in the case of in situ bioremediation. The remediated groundwater will be replaced eventu-

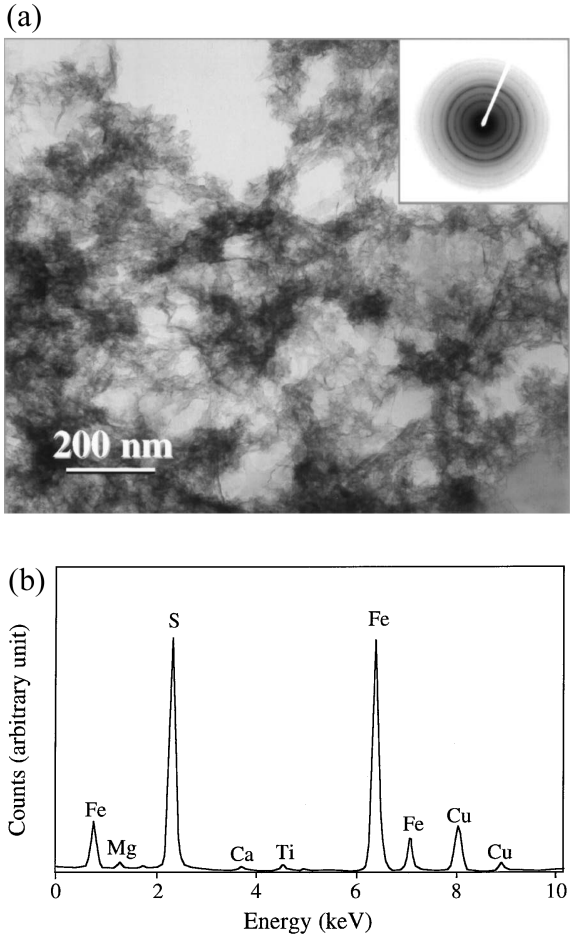

Fig. 6. (a) Mackinawite particles, electron diffraction pattern; and (b) chemical microanalysis spectrum of mackinawite (farm site).

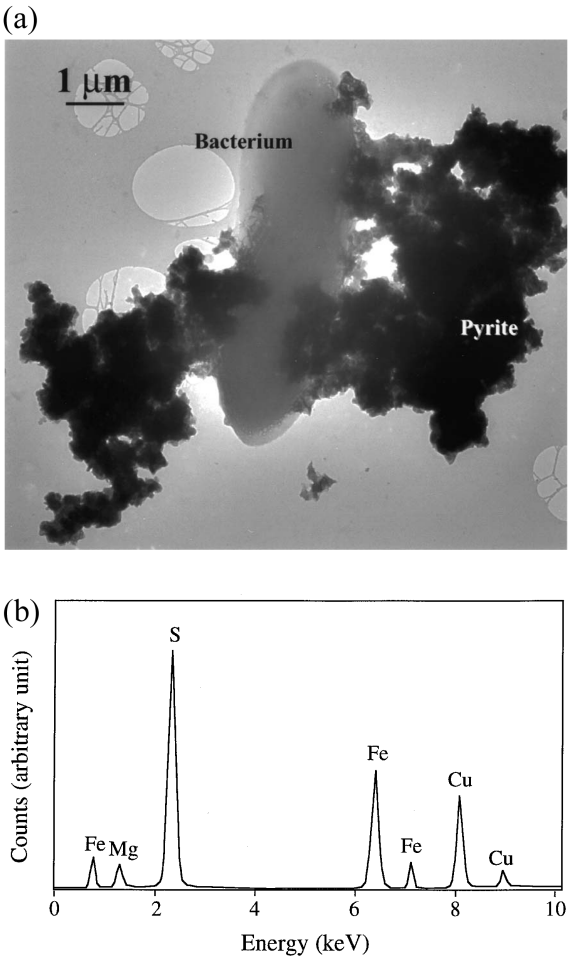

Fig. 7. (a) Bacterium with pyrite particles; and (b) chemical microanalysis of pyrite (mill tailings site, Germany).

ally by uncontaminated water containing oxygen, and uraninite could be reoxidized. We conducted batch and soil column experiments with the groundwater and soil from the mill tailings site at Tuba City and the biologically precipitated uraninite was leached with oxygen-rich uncontaminated groundwater from the same site (Abdelouas et al., 1999a). In the batch experiments we kept a constant molar ratio $UO_2/FeS_{0.9} = 1.5 \times 10^{-3}$ but we varied the oxygen supply between 7 and 58 μM. We found that the amount of oxidized uraninite increased with increasing amounts of oxygen supplied. While most of the oxygen (> 90%) was consumed by mackinawite oxidation, a small fraction of the oxygen (< 0.1%) was used to oxidize uraninite; the rest of the oxygen was consumed by oxidation of biomass. In the column experiments, the concentration of uranium in solution (outlet of the column) was on the order of a few μg/l, typically 4 μg/l, and did not change with time in the presence of mackinawite and dissolved oxygen. Again, it was found that most of the oxygen was consumed by makinawite oxidation. By using the inventory of uraninite and mackinawite in the column and the concentration of oxidized uranium and sulfide in the groundwater leaving the column, we calculated that before total oxidation of mackinawite all uraninite is expected to oxidize at a very slow rate. Hence, the large amount of iron sulfide (roughly 4.5 mM) in the column compared to that of uraninite (roughly 10^{-4} mM) protected uraninite from rapid oxidation and prevented the increase of U(VI) concentration above 44 μg/l, the groundwater protection standard in the United States. The preferential oxidation of mackinawite relative to uraninite was expected because the redox intensity $p\varepsilon^\circ$ of SO_4^{2-} reduction, $p\varepsilon^\circ = -3.75$ (Stumm and Morgan, 1981), is lower than that of U(VI), $p\varepsilon^\circ = +4.9$ (Abdelouas et al., 1998a). Rhodochrosite is not expected to protect uraninite from reoxidation because the redox intensity of Mn(IV), $p\varepsilon^\circ = +8.9$ (Stumm and Morgan, 1981), is higher than that of U(VI).

To study the effect of $UO_2/FeS_{0.9}$ on uraninite dissolution we conducted batch experiments where the oxygen concentration was kept constant at 0.4×10^{-2} mM and the molar ratio $UO_2/FeS_{0.9}$ was varied between 6.1×10^{-3} and 1.4×10^{-3} by varying the initial concentration of $FeSO_4 \cdot 7H_2O$ of the groundwater. The results are given in Fig. 8, which shows that U(IV) is oxidized to U(VI) whose concentration reaches a maximum in all experiments and decreases to below 20 μg/l after 23 days. Fig. 8 shows that the maximum concentration of U(VI) reached in each experiment increased with increasing the ratio $UO_2/FeS_{0.9}$. In other words, the more iron sulfide present, the higher the stability of uraninite. The slow decrease of U(VI) concentration over time is probably due to reduction and reprecipita-

tion of uraninite because, after consumption of oxygen, the redox intensity of the solution is determined by H_2S/HS^-.

Iron(III) compounds are ubiquitous in soils and sediments and their concentration is usually much higher than the small amount of precipitated uraninite. Iron(III) oxides and hydroxides are found in concentrations of a few percent depending on the origin of the soil (Langmuir, 1997). Sulfate concentrations are also often quite high. The median concentration of sulfate in uncontaminated groundwaters is 30 mg/l (Turekian, 1977). Sulfate concentrations in acid-mine waters, tailings waters, and waste waters, the contaminated sites for potential application of bioremediation technologies, can exceed 30 g/l (Langmuir, 1997). Thus, much more mackinawite and other iron sulfides are formed than uraninite. It has been shown that mackinawite can protect uraninite for hundreds of years (Abdelouas et al., 1999a) using an acceleration test. In the case of iron-poor soil and sulfate-poor groundwater, the addition of iron sulfate to the groundwater would help precipitate enough iron sulfide to protect uraninite from oxidation, at least to the extent necessary to keep the uranium concentration below 44 μg/l.

4. Summary and conclusion

Bacteria capable of reducing uranium can be found in groundwaters with different chemical composition. The uranium reducers are primarily sulfate-reducers and can be stimulated by addition of nutrients to groundwaters with high concentrations of sulfate. Ethanol together with tri*meta*phosphate yielded the highest rates of sulfate and uranium reduction. The uranium-reducers can also be stimulated in groundwater with low sulfate concentration. Addition of iron sulfate may be necessary in iron- and sulfate-poor groundwater/soil systems to precipitate enough iron sulfide to protect uraninite from reoxidation in oxygenated groundwaters.

The present results suggest that in situ bioremediation may find application to remediate uranium contaminated sites. An engineered process of U in situ bioremediation relies on two critical issues: (1) the presence of bacteria capable of reducing uranium; and (2) mixing of the contaminated water with the necessary additives to stimulate bacterial growth. For the first issue, the present work suggests that uranium reducers are ubiquitous in nature. The second issue is strictly technical and there are many solutions to this problem.

Though significant progress was made with U bioremediation, demonstration of the technology in the field is necessary to confirm the laboratory results. We conducted a small in situ experiment to test our technology and to study the mixing process, but only for biological denitrification of nitrate-contaminated groundwater at a site in Albuquerque, NM (USA). Nitrate was reduced to nitrogen within 5 days (Abdelouas et al., 1999c).

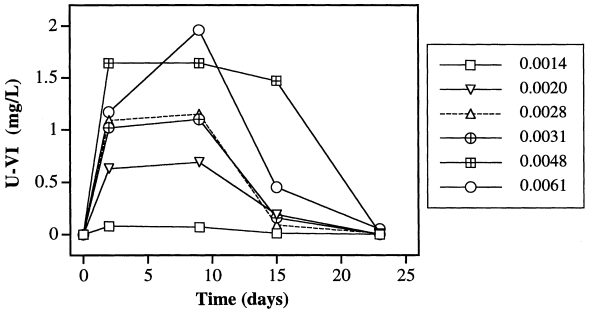

Fig. 8. Uraninite oxidation in oxygen-rich uncontaminated groundwater. Numbers in legend correspond to the uraninite/mackinawite molar ratio.

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