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Microbial removal of uranyl by sulfate reducing bacteria in the presence of Fe (III) (hydr)oxides

Yi Zhengji [a, b, *]

[a] Key Laboratory of Functional Organometallic Materials of Hengyang Normal University, College of Hunan Province, Hengyang, Hunan 421008, PR China
[b] State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang, Guizhou 550002, PR China

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ABSTRACT

Microbiological reduction of uranyl by sulfate reducing bacteria (SRB) has been proposed as a promising method for removal of radionuclide from groundwater. In this study, we examined the effect of two naturally occurring Fe(III) (hydr)oxides, hematite and goethite, on the bioreduction of U(VI) by a mixed culture of SRB via laboratory batch experiments. The biogenic precipitate from U(VI) bioreduction was determined using X-ray absorption near edge structure (XANES) analysis, showing a typical feature of uraninite (UO_2). In the presence of either hematite or goethite-containing Fe(III) ranging from 10 to 30 mM, the reduction of U(VI) was retarded by both minerals and the retardatory effect was enhanced with increasing amount of Fe(III) (hydr)oxide. When exposed to a mixture of hematite and goethite with the total Fe(III) kept constant at 20 mM, the retardatory effect on U(VI) reduction by the minerals were directly correlated with the fraction of hematite present. A slow increase in U(VI) concentration was also found in all Fe(III) (hydr)oxide treatments after 10–13 days, accompanied by the release of Fe(II) into the solution. The presence of Fe(III) (hydr)oxide can cause the eventual incomplete bioreduction of U(VI). However, it was not the case for the control without minerals. When mixing biogenic uraninite with hematite or goethite without SRB, Fe(II) was also detected in the solution. These findings suggest that the U(VI) remobilization after 10 ~ 13 days may be due to reoxidation of the uraninite by the solid-phase Fe (III) (hydr)oxide.

1. Introduction

Large amounts of uranium-bearing wastewater are generated in China and aboard every year during the mining, extraction, and processing of uranium for nuclear fuel and weapons. For instance, the Erqier uranium tailings impoundment as the largest one in South China has operated for almost forty years, radioactive nuclides have resulted in the serious contamination of the surface water and groundwater (Ma and Wang, 2000; Xie et al., 2005). Especially after the in situ leaching (ISL) uranium mining process was completed, the residue uranyl which cannot be pumped out from the aquifer will form a contaminant plume posing a serious threat to downstream groundwater resources. Long-lived radionuclides of ^{238}U series are hazardous substances because of their chemical toxicity and radioactivity. Once uranium was released into the environment, it can eventually reach the top of the food chain and be ingested by humans, causing kidney damage in high dose (Kurttio et al., 2006). The World Health Organization determined that U(VI) is a human carcinogen and recommended that the concentration in drinking water should be below 15 μg l^{-1}. Traditional ex situ remediation approaches based on pump and treat practice, such as lime neutralization, anion exchange, activated aluminum and biosorption, are not only prohibitively expensive but can also be limited by poor extraction efficiency, inhibitory competing ions and massive waste production. Besides, bringing the radioactive contaminants up to the surface can increase health and safety risks for cleanup workers and the public. Therefore, there is a great need for cost-effective alternatives to prevent its further migration and spread through the deep subsurface.

In recent years, the finding that sulfate reducing bacteria (SRB) can enzymatically convert soluble uranyl (U(VI)) to highly insoluble uraninite (U(IV)), a geochemically inactive form, has sparked interest in in situ bioremediation of uranium-contaminated groundwater (Gregory and Lovley, 2005; Renshaw et al., 2005; Vrionis et al., 2005). However, previous work mostly focused on isolation, identification of U(VI)-reducing microorganisms and elucidating mechanisms of U(VI) bioreduction processes (Elias et al., 2004; Nadia et al., 2004). To our knowledge, although

* Tel.: +86 734 8466055.
E-mail address: yizhengji2004@126.com

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direct microbial reduction of U(VI) is known, only limited investigations have been conducted on certain key environmental factors, such as presence of Fe(III) oxide minerals, on the process of U(VI) bioreduction (Sani et al., 2004). Moreover, such experiments were carried out using pure cultures (Gu and Chen, 2003; Senko et al., 2007; Komlos et al., 2008; Zhang et al., 2009). In contrast, easily available mixed cultures of bacteria have an advantage over pure cultures for in situ bioremediation since in operational biotreatment of large volumes of water, it is difficult to maintain culture purity due to the ubiquity of microorganisms in the environment. It has been demonstrated that mixed SRB are readily adaptable to polluted environmental conditions, are less liable to mutate and to be contaminated from other microorganisms, and are capable of more completely oxidizing carbon sources to form reducing conditions (Gibert et al., 2002). Therefore, mixed microorganisms predominant by SRB were used in our research (Spear et al., 2000).

Considering that the processes associated with microbiological reduction of U(VI) can have great impact on reducing uranium contamination in groundwater, many related factors and effects need to be investigated. It should also be pointed out that Fe(III) (hydr)oxides as ubiquitous oxidants in nature are mainly responsible for bacterially promoted oxidation of organic compounds in subsurface environments (Liu et al., 2009). In uranium-contaminated groundwater, the coexisting insoluble Fe(III) (hydr)oxides, are recognized as multiple electron acceptors of metal reducing bacteria which may be likely associated with respiration. Also, solid-phase Fe(III) is commonly present in much higher concentrations than U(VI). Therefore, it is hard to estimate the fate of U(VI) in Fe(III) (hydr)oxides-bearing environments as bacterial U(VI) reduction occurs. In this paper, we examined the effect of two Fe (III) oxides, hematite and goethite, on the bacterial U(VI) reduction. Evaluation of their effects will be helpful to better understand interactions of contaminants with bacteria and mineral phases in the subsurface as well as to develop bioremediation strategies for uranium-contaminated groundwater.

2. Materials and methods

2.1. Chemical reagents and source of microorganisms

2.1.1. Chemicals

Hematite (α–Fe_2O_3 powder; ca. 8.5 $m^2\ g^{-1}$) was purchased from Sinopharm Chemical Reagent Company (Shanghai, China). Goethite (α–FeOOH powder; ca. 55.6 $m^2\ g^{-1}$) 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 (Li et al., 2006). The specific surface areas of two oxides were determined by the BET method with nitrogen adsorption using the Surface Area Analyzer (ASAP-2000, Micromeritics). The synthetic goethite was washed free of salt by dialysis, freeze-dried and passed through a 100 mesh sieve. Synthetic goethite was measured by X-ray diffraction (XRD) and exhibited no evidence of crystalline impurities. The above Fe(III) (hydr)oxides were not autoclaved due to potential phase modifications that can occur during autoclaving. $UO_2Cl_2 \cdot 3H_2O$ was purchased from Hongyue reagent company (Hengyang, China). The U(VI) stock solution containing 1 mg ml^{-1} U was prepared using deaerated water in a glovebox.

2.1.2. Cell culturing and harvesting

Black activated sludge was taken a with a sludge sampler (Ballcheck KB, Eijkelkamp, Holland) from a ditch near an abandoned uranium mill tailings impoundment in Hengyang, China. This is the largest uranium milling tailings disposal site in China, covering 170 ha and containing 18.8 million tonnes of tailings. The sediment sludge sample was transferred to a Mason jar until it was completely full and then the jar was sealed tightly to avoid any direct contact with oxygen. At the time of sampling (17 June, 2005), the sludge was black and the odor of H_2S was detectable, indicating that anaerobic condition was prevalent. The sediment was stored at 4 °C up until the experimentation. Continuous cultivation and enrichment of the mixed SRB was carried out immediately after transporting the sample into our laboratory. Ten ml sediment sludge was transferred into a 250 ml serum bottle containing 100 ml sterilized culture medium as well as approximately 50 mg l^{-1} U (VI). The medium was modified from Postgate C medium and consisted of (per litre with distilled water): 0.5 g KH_2PO_4; 1.0 g NH_4C1; 1.0 g yeast; 0.1 g $CaCl_2 \cdot 6H_2O$; 2.0 g $MgSO_4 \cdot 7H_2O$; 4.5 g Na_2SO_4; 0.002 g $FeSO_4 \cdot 7H_2O$; 5.0 ml sodium lactate (70% w/v) (Postgate, 1984). Resazurin (1 mg l^{-1}) was added as a redox indicator to show any contamination by molecular oxygen. Cysteine (3.0 g l^{-1}) was added to reduce the trace amount of oxygen remaining in the medium after autoclaving. The pH was adjusted to 7.0 with 50% NaOH (w/v). The headspace of the serum bottles was pressurized with ultrapure nitrogen, then capped with butyl rubber septa and crimped with an aluminum seal. The above procedures were performed inside a glovebox (Mikrouna Inc. in China). All chemicals utilized for these studies were reagent grade or better without further purification.

The inoculated serum bottles were then put into a rotary-shaker (150 rpm at 35 °C) in the dark. After a week of incubation, the formation of black precipitate on the bottoms and walls of serum bottles indicated growth of SRB. Then 10 ml enrichment culture was taken and transferred into 100 ml fresh culture medium amended with approximately 50 mg l^{-1} U(VI) according to the procedures described above, and the second enrichment culture was established. During the following two weeks, the third and fourth enrichment transfer cultures were initiated successively in a similar manner. Finally, mixed bacterial cells were harvested by centrifugation (6000 g, 15 min) and washed twice in fresh, anoxic $NaHCO_3$ buffer (2.5 g l^{-1}) under an extra pure N_2 atmosphere. The concentrated mixed SRB cells were resuspended in a serum bottle containing fresh, anoxic $NaHCO_3$ buffer (2.5 g l^{-1}) to give a final concentration of approximately 5×10^9 cells ml^{-1}. These mixed SRB cell suspensions were sealed in serum bottles fitted with thick butyl rubber stoppers under extra pure N_2 atmosphere and used as inocula for the following quantification and U(VI) bioredution experiments. The modified Postgate C medium was primarily used to increase SRB numbers; the enumeration and identification of SRB with different morphological types were conducted using standard methods as previously described (Ren and Wang, 2004). Since the inocula were cultured and obtained anaerobically, other possible anaerobes (metabolically interdependent), including methane producing bacteria (MPB), denitrifying bacteria (DNB) and acetogenic bacteria (AB) were also determined by standard most probable number (MPN) methods (Li et al., 1996).

2.2. Bacterial reduction experiments

All U(VI) reduction experiments were conducted anaerobically in a series of 250 ml serum bottles in the dark, each containing 150 ml test solution. Generally, the test solutions contained lactate as an electron donor, U(VI) and sulfate as electron acceptors and the two above-mentioned minerals. The initial concentrations of lactate, U(VI) and sulfate were maintained at 3 g l^{-1}, 40 mg l^{-1} and 2 g l^{-1} respectively. The amount of hematite and goethite added to the solutions ranged from 0 to 30 mg l^{-1}. Cysteine (3.0 g l^{-1}) was added to reduce the trace amount of oxygen remaining in the solutions after autoclaving. It should be noted that the test solutions did not contain vitamins, organic matter and other nutrients in order to limit SRB growth and eliminate their possible interference with U(VI) bioreduction. After all components were added, the serum bottles were purged with extra pure N_2 for 15 min and then sealed with butyl rubber stoppers. Aliquots of the mixed SRB cells were injected into each serum bottle of test solution using a syringe to achieve a final concentration of 2×10^8 cells ml^{-1} after three days of incubation. Serum bottles with no cells, no minerals or no sulfate were used as controls. The aforementioned serum bottles containing U(VI), inocula and minerals were shaken horizontally at 35 °C for four weeks, while cells were metabolically active. Samples were taken regularly using a N_2-purged syringe fitted with a needle and then filtered through 0.22 μm membranes. The concentrations of U(VI), soluble sulfide and Fe(II) were determined in the filtrates. A kinetic phosphorescence analyzer (KPA-11, Chemchek Instrument, Richland, WA) was used to measure U(VI) concentration (Bernad et al., 2005). This method is specific for analyzing all forms of U(VI), with a detection limit $\leq$0.1 mg l^{-1} and a standard deviation of ca. 5%. The soluble Fe(II) and soluble sulfide concentration was determined spectrophotometrically (Behrends and Van Cappellen, 2005).

2.3. Abiotic experiments with biogenic UO_2(s) and Fe(III) (hydr)oxide

To determine the possibility of reoxidation of biogenic uraninite by Fe(III) (hydr)oxide, separate abiotic experiments without bacteria were also conducted. Briefly, collected black precipitates from U(VI) bioreduction were incubated with two concentrations of hematite ranging from 50 to 100 mM. The initial precipitate concentration was 40 mg l^{-1} (w/v). As a control, $FeCl_3$ solution as aqueous Fe (III) was also added into uraninite suspension in a glovebox under N_2 atmosphere, making the final aqueous Fe(III) up to 50 mM. Over the course of an experiment aliquots of the reaction medium were periodically retrieved with a syringe. The U(VI) concentration in the solution was also determined as described in Section 2.2.

2.4. X-ray absorption near-edge Spectroscopy (XANES) analysis

In order to evaluate if U(VI) was truly reduced to U(IV) species, the black precipitate on the serum bottle bottom was collected for microprobe-XANES spectroscopic analysis (National Synchrotron Radiation Laboratory, Hefei). Synthetic U (IV)O_2 (uraninite) and U(VI)$O_3 \cdot 2H_2O$ served as reference standards. The

experimental sample and the two standards were prepared by mounting submilligram quantities in a square hole in a thin plastic coupon which was then sealed on both sides with Kapton tape. During XANES analysis, the detector chamber was fitted with a Strontium filter and was continuously flushed with Helium gas to prevent oxidation of the samples by air. The resulting spectra were normalized for comparative purposes.

3. Results and discussion

3.1. Characterization of the mixed cell inocula used in the U(VI) bioreduction experiment

The most probable number (MPN) analysis of the inocula showed that the quantity of SRB was much greater than the other metabolically interdependent anaerobes, methane producing bacteria (MPB) and denitrifying bacteria (DNB) (Table 1). The density of DNB was limited due to the shortage of nitrate acceptors during sludge acclimatization in the modified Postgate C medium, which was chiefly appropriate for SRB growth. The SRB consisted of at least five different genera, *Desulfovibrio* spp. were dominant with the total number exceeding 10^7 cells ml^{-1}, the numbers of *Desulfococcus* and *Desulfosarcina* spp. were negligible. Preliminary experiments suggest that our mixed culture could precipitate U(VI) at a high rate under laboratory conditions. This may be attributed to two dominant species *Desulfovibrio desulfuricans* and *Desulfovibrio vulgaris*, which are capable of catalyzing reduction of U(VI) and growing with U(VI) as the terminal electron acceptor as well (Lovley and Phillips, 1992; Lovley et al., 1993). During incubation, black precipitate was detected on the bottom of serum bottles. The precipitate was analyzed by XANES and compared to standard U (IV) and U(VI) XANES spectra, and the result indicates that the precipitate exhibited features of the U(IV) standard and contained 91% uranium as U(IV) (Fig. 1).

3.2. Microbial U(VI) reduction in the presence of different ratios of Fe(III) (hydr)oxide

In the two mineral-free controls with or without viable cells, the U(VI) concentration decreased slightly during the first 3 d before cell inoculation (Fig. 2). The initial slight decreases in U(VI) levels may be due to the adsorption of uranyl cations onto the surface of serum bottles. Whereas in those bottles containing minerals, significant decreases of U(VI) concentrations were observed at early stage before cell inoculation. Besides, the decline rate of U(VI) level for hematite-containing group was slower in comparison to goethite-containing group (Fig. 2). These results indicate that both minerals have adsorption properties. The better adsorption performance of goethite was presumably associated with its higher surface area. Redden et al. (2001) reported that iron oxides had strong ability to adsorb uranyl cations.

After 3 d, five of the six treatments were inoculated with SRB cells. The U(VI) concentration remained nearly unchanged for the control without both minerals and cells. However, there was appreciable change of U(VI) concentration for the control without minerals. Over 95% U(VI) was reduced by the end of the experiment (Fig. 2). Obviously, the abrupt decrease of U(VI) concentration for mineral-free control was attributed to microbial activity. It was also found that the U(VI) removal rate of four mineral-containing groups was slower than that of the mineral-free control. This result suggests that the presence of hematite or goethite may inhibit the process of U(VI) reduction. Interestingly, the U(VI) concentrations of four mineral treatments all dropped to the lowest points and then gradually increased after 10–13 d, yet this was not the case for the mineral-free control. With regard to three hematite treatments, the increase rate of the soluble U(VI) levels at late stage was associated with the content of hematite initially present. The higher the initial level of hematite, the higher the final U(VI) concentration reached (Fig. 2).

The concentration change of soluble sulfide during U(VI) bioreduction was also determined to monitor microbiological activity (Fig. 3). Apparently, SRB cells kept active throughout the experiment. No decrease in sulfide concentration was found in the control without minerals. However, the concentration of sulfide increased during the first 10–13 d accompanied with microbial reduction of U (VI) for the mineral-bearing bottles. After U(VI) was nearly completely removed from the solution, the sulfide levels declined.

Table 1
Composition of the microorganisms used in the U(VI) bioreduction experiment.

Microorganisms	Cell number (ml^{-1})
Denitrifying bacteria (DNB)	7.61×10^3
Acetogenic bacteria (AB)	Not detected
Methane Producing Bacteria (MPB)	3.07×10^4
Sulfate reducing bacteria (SRB)	4.54×10^9
Desulfovibrio(D. desulfuricans, D. vulgaris)	10^7–10^9
Desulfobulbus (D. elongates, D. propionicus)	10^4–10^6
Desulfobacter (D. multivorans)	10^3–10^4
Desulfobacterium (D. autotrophicum, D. vacuolatum)	10^2–10^4
Desulfococcus (D. Posgatei)	10^1–10^3
Desulfotomaculum (D. nigrificans, D. orientis)	10^1–10^3
Desulfosarcina (D. variabilis)	10^1–10^2

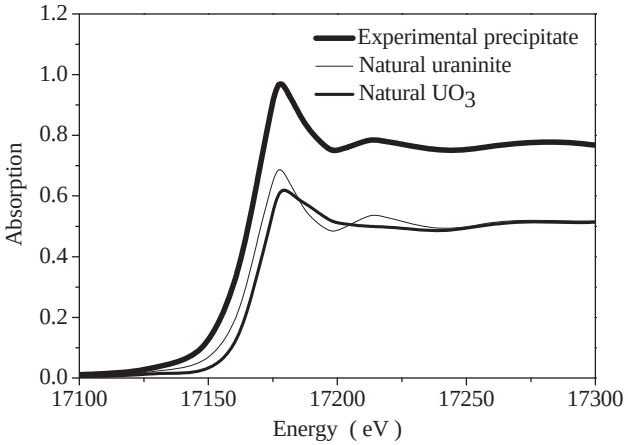

Fig. 1. XANES spectra for collected black precipitate and for U(VI) standards from UO_2 and $UO_3 \cdot 2H_2O$.

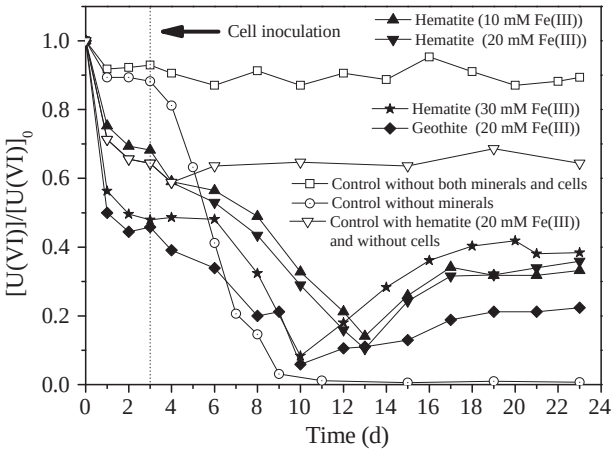

Fig. 2. Effect of Fe(III) (hydr)oxide (hematite and geothite) on U(VI) bioreduction by mixed SRB $[U(VI)]_0$ represents the initial U(VI) concentration in serum bottles (40 mg l^{-1}); [U(VI)] represents the U(VI) concentration at any time in serum bottles.

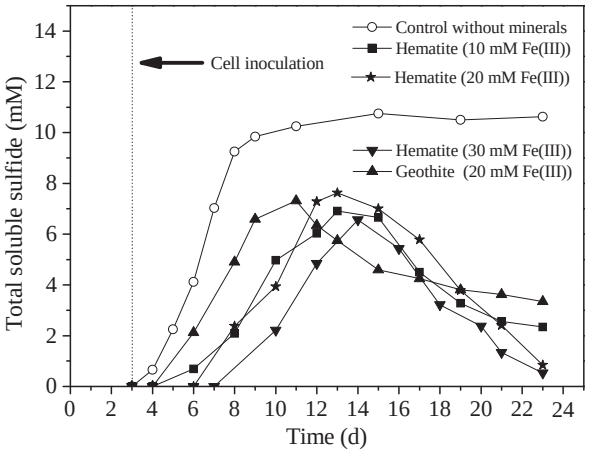

Fig. 3. The soluble sulfide produced during U(VI) bioreduction in the presence of Fe(III) (hydr)oxides.

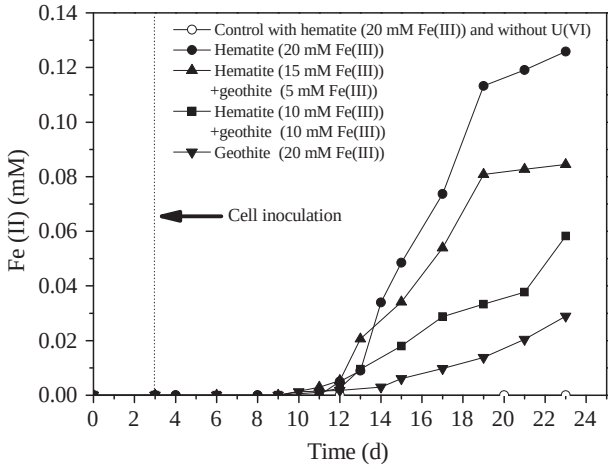

Fig. 5. Fe(II) released from Fe(III) (hydr)oxides during U(VI) bioreduction.

This phenomenon may be explained that aqueous Fe(II) or solid-phase Fe(III) was capable of eliminating sulfide. During the incubation, black precipitates were detected at the bottom of all bottles, most likely forming insoluble ferrous sulfide and uraninite.

3.3. Microbial U(VI) reduction in the presence of different ratios of hematite and geothite

When varying ratios of hematite to geothite were induced into cell suspensions (total Fe(III) was kept constant at 20 mM), the enzymatic reduction process was strongly inhibited compared with the mineral-free control (Fig. 4). The degree of inhibition produced by the mixed minerals depended upon the fraction of hematite present. The concentration of U(VI) remaining in solution for mineral-free control was ca. 1.5–2 times the levels of two groups containing both hematite and geothite at the end of 7 d (Fig. 4). After 9 d, over 95% U(VI) was removed from solution for the mineral-free control. However, the U(VI) removal efficiency just achieved 78.4% and 85.9% when the hematite Fe(III) fraction was 75% and 50% respectively. It should be noted that U(VI) can still be effectively removed although sulfate was not added in this experiment. This result suggests that U(VI) was directly reduced enzymatically, rather than indirectly by H_2S produced during dissimilatory sulfate reduction. This is consistent with N'Guessan's result that U(VI) reduction may occur through electron transfer from electron donor to U(VI) as an electron acceptor, in which sulfidogenic activity is not involved U(VI) reduction (N'Guessan et al., 2008).

Similar to Fig. 2, the U(VI) concentration for mixed mineral-containing groups not only increased gradually but also related to the fraction of hematite at late stage ca. after 10 d (Fig. 4). Surprisingly, the elevation of U(VI) level was accompanied with the release of Fe(II) into solution (Fig. 5). In the control experiment directly incubating hematite and cells in the absence of U(VI), no Fe (II) was detected in the solution. This finding further corroborated that Fe(II) generation here was not likely the result of microbial reduction of Fe(III) (hydr)oxides. To date the discovered SRB spp. which can utilize solid-phase Fe(III) as terminal an electron acceptor are extremely limited. Hence we speculate that the increase of both Fe(II) and U(VI) at late stage was due to the redox reaction between the biogenic uraninite and Fe(III) (hydr)oxides. In other words, the bioreduced U(IV) in this system could readily be reoxidized or resolubilized into solution after depletion of all lactate organic matter.

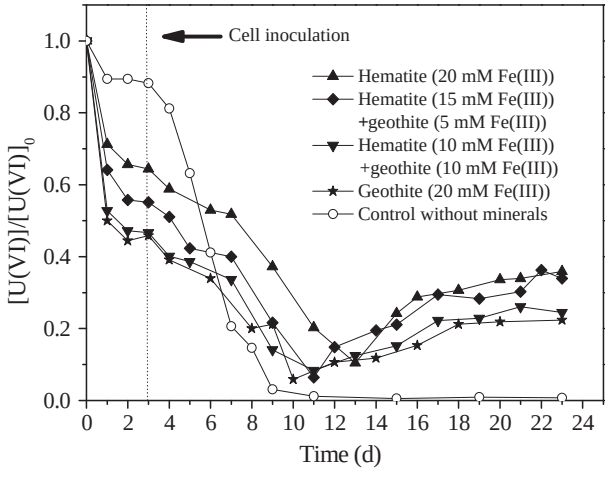

Fig. 4. U(VI) bioreduction in the presence of different ratios of hematite and geothite (no sulfate in medium). $[U(VI)]_0$ represents the initial U(VI) concentration in serum bottles (40 mg l^{-1}); [U(VI)] represents the U(VI) concentration at any time in serum bottles.

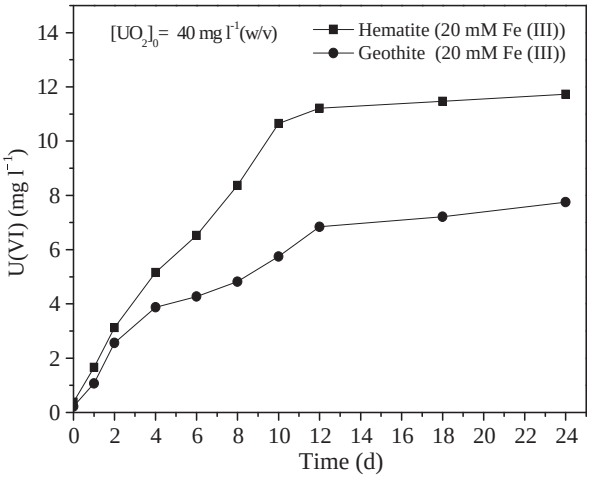

Fig. 6. Oxidation of biogenic uraninite to U(VI) by Fe(III) (hydr)oxides.

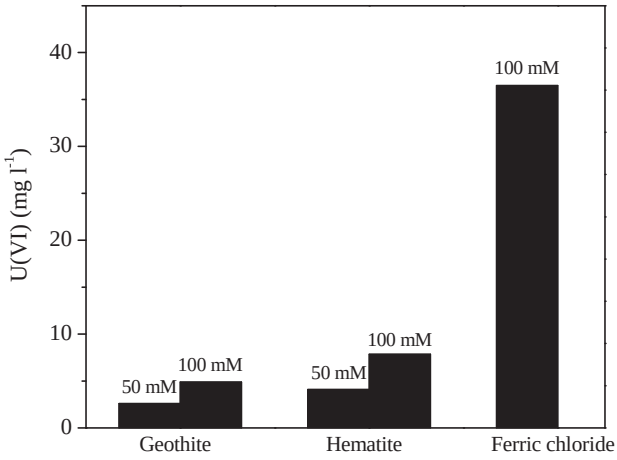

Fig. 7. Reoxidation of 40 mg l^{-1} (w/v) biogenic uraninite by hematite, goethite and ferric chloride. The incubation time was 1 d. The top of the column bar showed the solid-phase Fe(III) content of iron oxide or the aqueous ferric cations added into the solution.

3.4. Oxidation of biogenic uraninite by Fe(III) (hydr)oxides and aqueous Fe(III)

In order to further elucidate the reoxidation of bioreduced U(IV) by solid-phase Fe(III), abiotic experiments were conducted through incubating two above Fe(III) (hydr)oxides with biogenic uraninite respectively. The results demonstrate that both hematite and geothite showed an initial rapid oxidation of U(IV) to soluble U(VI) during the first 4 d, followed by a slower rate of oxidation. The rate of oxidation was more rapid in the presence of hematite than in the presence of geothite (Fig. 6). When UO_2 was incubated with two levels of hematite ranging from 50 to 100 mM, the oxidation of UO_2 was clearly promoted by increasing concentrations of hematite, and the higher amounts of U(IV) oxidation corresponded directly to higher concentrations of hematite. Moreover, it was also found that U(IV) were more effectively oxidized by aqueous Fe(III) within 1 d in contrast to hematite treatments (Fig. 7). Similar observations have been reported using a pure culture '*Desulfovibrio Desulfuricans* G20' previously (Sani et al., 2004). It appears that the oxidation of uraninite by Fe(III) (hydr)oxide is a slow and incomplete process compared with aqueous Fe(III). The above discussion implied that U (IV) can potentially serve as an electron donor to reduce solid-phase Fe(III) present in Fe(III) (hydr)oxides. We speculate that the possible reaction between uraninite and two solid-phase Fe(III) oxides as well as aqueous Fe(III) can be described as Eqns. (1)–(3) in Table 2.

Using these equations, free standard energies ($\Delta G^{\circ\prime}$) at pH 7.0 were calculated (Fu, 1990; Thauer et al., 1977; Ye and Hu, 2002). Obviously, their $\Delta G^{\circ\prime}$ values are negative implying that three reactions may occur thermodynamically. Moreover, the uraninite oxidation by hematite releases three times more free energy than by goethite, indicating that hematite has an advantage over geothite in competing for uraninite as an electron donor. The calculation results explain why the rate of uraninite oxidation was more rapid in the presence of hematite than in the presence of goethite. Likewise, the Eqn. (3) has a large negative $\Delta G^{\circ\prime}$ value implying that the forward reaction can proceed spontaneously and completely. From the above analysis, it is quite evident that the presence of Fe(III)-(hydr)oxides may reverse the process of U(VI) bioprecipitaion based on their redox interaction with uraninite.

Table 2
Free energies of reactions coupling the oxidation of uraninite to the reduction of solid-phase and aqueous Fe(III).

Equation (redox reaction)	Standard free energy change
$UO_2(s) + Fe_2O_3(s) + 6H^+ \rightarrow UO_2^{2+} + 2Fe^{2+} + 3H_2O$ (1)	$\Delta G^{\circ\prime} = -248.4$ kJ mol^{-1}
$UO_2(s) + 2FeOOH(s) + 6H^+ \rightarrow UO_2^{2+} + 2Fe^{2+} + 4H_2O$ (2)	$\Delta G^{\circ\prime} = -76.5$ kJ mol^{-1}
$UO_2(s) + 2Fe^{3+} \rightarrow UO_2^{2+} + 2Fe^{2+}$ (3)	$\Delta G^{\circ\prime} = -621.7$ kJ mol^{-1}

4. Conclusions

The potential for bacterial reduction of U(VI) to highly insoluble form U(IV) by a mixed culture of SRB in the presence of two naturally ubiquitous Fe(III) (hydr)oxides, hematite or goethite, was investigated. The results suggest that the process of U(VI) bioreduction was retarded by both minerals. Besides, the retardatory effect was increased with increasing level of hematite or goethite. When a mixture of hematite and goethite (the total Fe(III) was constant but ratios of two minerals was varied) was added, the retardatory effect on uranyl bioreduction was highly dependent on the fraction of hematite present. The higher the hematite amount, the slower the uranyl bioreduction. The bioreduced U(VI) was partly resolubilized for all Fe(III) (hydr)oxide treatments, accompanied with the release of Fe(II) into the solution. Moreover, the amount of Fe(II) released into solution was also directly related to the proportion of the hematite present. The U(VI) remobilization at late stage might be attributed to reoxidation of the uraninite by the solid-phase Fe(III) (hydr)oxide. So complete removal of all available solid-phase Fe(III) is the prerequisite that U(VI) can be completely removed. Our results have important implications for field application of in situ bioremediation. Considering that the presence of Fe (III)-(hydr)oxides may limit in situ U(VI) immobilization by SRB, it is required that Fe(III) reducing bacteria should be first employed to exhaust all available solid-phase Fe(III).

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