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Uranium reduction and resistance to reoxidation under iron-reducing and sulfate-reducing conditions

*Benjaporn Boonchayaanant, Dipti Nayak, Xin Du, Craig S. Criddle**

Department of Civil and Environmental Engineering, Stanford University, Stanford, CA, USA

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ABSTRACT

Oxidation and mobilization of microbially-generated U(IV) is of great concern for in situ uranium bioremediation. This study investigated the reoxidation of uranium by oxygen and nitrate in a sulfate-reducing enrichment and an iron-reducing enrichment derived from sediment and groundwater from the Field Research Center in Oak Ridge, Tennessee. Both enrichments were capable of reducing U(VI) rapidly. 16S rRNA gene clone libraries of the two enrichments revealed that *Desulfovibrio* spp. are dominant in the sulfate-reducing enrichment, and *Clostridium* spp. are dominant in the iron-reducing enrichment. In both the sulfate-reducing enrichment and the iron-reducing enrichment, oxygen reoxidized the previously reduced uranium but to a lesser extent in the iron-reducing enrichment. Moreover, in the iron-reducing enrichment, the reoxidized U(VI) was eventually re-reduced to its previous level. In both, the sulfate-reducing enrichment and the iron-reducing enrichment, uranium reoxidation did not occur in the presence of nitrate. The results indicate that the *Clostridium*-dominated iron-reducing communities created conditions that were more favorable for uranium stability with respect to reoxidation despite the fact that fewer electron equivalents were added to these systems. The likely reason is that more of the added electrons are present in a form that can reduce oxygen to water and U(VI) back to U(IV).

1. Introduction

Uranium mining and processing for nuclear weapon production has resulted in extensive uranium contamination of soil and groundwater at U.S. Department of Energy (DOE) sites. While several options are available for remediating uranium contaminated sites, in situ bioremediation is attractive because it costs less compared to pump-and-treat methods and does not require off-site handling of hazardous materials (McCullough et al., 1999). Many microorganims, including sulfate-reducing bacteria, iron-reducing bacteria, and fermenting bacteria, are known to reduce mobile U(VI) to sparingly soluble uraninite, UO_2(s) (Lovley et al., 1991, 1993a, b; Lovley and Phillips, 1992; Gorby and Lovley, 1992; Francis et al., 1994; Anderson and Lovley, 2002). Abiotic reductive processes mediated by some of the reduced products of microbial metabolism, such as hydrogen sulfide and ferrous iron, also produce insoluble U(IV) (Hua et al., 2006; O'Loughlin et al., 2003; Boonchayaanant et al., in press). Thus, biostimulation of U(VI) reduction is one potential method to immobilize uranium (Anderson and Lovley, 2002).

A few pilot studies have been conducted at uranium-contaminated sites to investigate the potential of in situ reductive bioremeditation (Anderson et al., 2003; Wu et al., 2006a, b). One of these tests was performed in Area 3 of the

* *Corresponding author*. 473 Via Ortega, Yang and Yamazaki Environment and Energy Building 151, Stanford, CA 94305, USA. Tel.: +1 650 723 9032; fax: +1 650 725 3164.
E-mail address: ccriddle@stanford.edu (C.S. Criddle).
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DOE Natural and Accelerated Bioremediation Research Field Research Center (FRC), Oak Ridge, Tennessee, adjacent to the former S-3 ponds (Wu et al., 2006a, b, 2007). In this pilot-scale test, aqueous U(VI) concentrations below the US EPA drinking water standard (<0.126 μM) were achieved in situ; and the bioreduced/immobilized U(IV) was stable under anaerobic conditions (Wu et al., 2007). This suggests the potential effectiveness of full-scale bioremediation at uranium-contaminated sites.

While the above studies are promising, reoxidation of bioreduced U(IV) has been observed in the presence of oxygen or nitrate in laboratory and field experiments (Abdelouas et al., 1999; Finneran et al., 2002; Senko et al., 2005; Moon et al., 2007; Wu et al., 2007). In the pilot scale test at Oak Ridge Field Research Center (FRC), Oak Ridge, TN, introduction of dissolved oxygen led to reoxidation and remobilization of uranium (Wu et al., 2007). Strategies to control reoxidation are needed for reliable immobilization of uranium.

U(VI) reduction has been observed under both sulfate-reducing and iron-reducing conditions. Both sulfate-reducing bacteria (SRB) and iron-reducing bacteria (FeRB) reduce U(VI) to U(IV) enzymatically; they also produce hydrogen sulfide and Fe(II) species which can themselves reduce U(VI) (Hua et al., 2006; O'Loughlin et al., 2003; Boonchayaanant et al., in press). The U(IV) minerals generated by these abiotic and biotic reductants may differ in their susceptibilities to reoxidation and remobilization (Senko et al., 2007), and reduced species of sulfur and iron may function as reducing buffers, preventing oxidation of U(IV) (Moon et al., 2007; Senko et al., 2005). Until now, however, there have been no controlled experimental studies comparing uranium reoxidation under iron-reducing and sulfate-reducing conditions given the addition of similar levels of reducing power. Such studies are needed to enable the rational design and operation of effective bioremediation schemes.

Accordingly, the primary objective of this study was to directly compare reoxidation of uranium in sulfate-reducing and iron-reducing enrichments after exposure to oxidants when the electron equivalents added to both systems are similar and the electron acceptors are present in excess.

2. Materials and methods

2.1. Sulfate-reducing enrichment

2.1.1. History of the enrichment

The original source of microorganisms for sulfate-reducing enrichments was sediment and groundwater from monitoring well FW102-2 at the FRC in Oak Ridge, Tennessee. Mixed sediment and groundwater (~3% v/v) was used to initiate five ethanol-fed sulfate-reducing enrichments. The growth medium is described below. The resulting enrichments were grown anaerobically at room temperature (22 °C) for 16 days then pooled and used to inoculate sulfate-reducing enrichments designed to assess growth and reoxidation.

The growth medium contained: Na_2SO_4, 0.83 g/l; ethanol, 0.46 g/l; $NaHCO_3$, 0.42 g/l; NaCl, 0.9 g/l; KCl, 0.5 g/l; NH_4Cl, 1 g/l; $MgCl_2 \cdot 6H_2O$, 0.2 g/l; $CaCl_2 \cdot 2H_2O$, 0.016 g/l; $Na_3P_3O_9$, 0.1 g/l; $Na_2S \cdot 9H_2O$, 0.22 g/l; vitamin stock solution I, 1 ml/l; vitamin stock solution II, 1 ml/l; and trace element stock solution, 10 ml/l. Vitamin stock solution I consisted of hydroxocobalabin hydrochloride, 0.05 g/l; *p*-aminobenzoic acid, 0.2 g/l; biotin, 0.1 g/l; nicotinic acid, 0.35 g/l; L-pantothenic acid-hemicalcium salt, 0.1 g/l; pyrodoxin monohydrochloride, 0.1 g/l; and thiamin hydrochloride, 0.3 g/l. Vitamin stock solution II consisted of folic acid-$2H_2O$, 0.002 g/l; riboflavin, 0.005 g/l; and OL-6,8-thiocitic acid, 0.005 g/l. The trace element stock solution consisted of $ZnSO_4 \cdot 7H_2O$, 0.1 g/l; $MnSO_4 \cdot H_2O$, 0.085 g/l; boric acid, 0.06 g/l; $CoCl_2 \cdot 6H_2O$, 0.02 g/l; $CuCl_2$, 0.004 g/l; $NiSO_4 \cdot 6H_2O$, 0.028 g/l; $Na_2MoO_4 \cdot 2H_2O$, 0.04 g/l; and $FeCl_2$, 0.3 g/l. Resazurin, 0.002 g/l, was added to serve as a redox indicator. The medium (100 ml medium in 150 ml serum bottle) was prepared anaerobically under helium headspace. The pH was adjusted to 6.3 ± 0.1 using HCl.

2.1.2. Sulfate-reducing enrichment growth in the absence of uranium

Five replicate cultures, seeded with the pooled inoculum as described above, were grown anaerobically at room temperature (22 °C) for 11 days. Liquid samples (1.7 ml) were periodically collected for measurement of ethanol, acetate, and hydrogen sulfide. Gaseous samples (200 μl) were collected for hydrogen measurement. After 11 days of growth, liquid samples (8 ml) were collected for protein analysis.

2.1.3. U(VI) reduction and reoxidation in sulfate-reducing enrichment

Six replicate cultures (106 ml culture in a 150 ml serum bottle), seeded with the pooled inoculum described above, were grown anaerobically at room temperature (22 °C). The initial concentration of ethanol was 10.1 ± 1.5 mM. Liquid samples (1.7 ml) were periodically collected for measurement of ethanol, acetate, and hydrogen sulfide. Gaseous samples (200 μl) were collected for measurement of hydrogen. After six days of growth, the pH was re-adjusted to 6.3 ± 0.1. Uranyl acetate ($UO_2(CH_3COO)_2$) (0.65 ml of 15.47 mM) was then added to a final concentration of 114 μM. U(VI) concentrations were periodically assayed thereafter. After two days of U(VI) reduction, 70 ml headspace gas was removed from three of the replicate cultures and replaced by 70 ml of air (1 atm), thus exposing these enrichments to 14.7 ml of O_2 (0.71 mmoles O_2). Simultaneously, the three remaining enrichments were exposed to 6 mM nitrate, added as sodium nitrate. Both the oxygen-exposed and nitrate-exposed enrichments were then monitored for U(VI), ethanol, acetate, hydrogen sulfide, and hydrogen.

2.2. Iron-reducing enrichment

2.2.1. History of the enrichment

The original source of microorganisms for the iron-reducing enrichments was sediment and groundwater from monitoring well FW102-2 at the Oak Ridge FRC, the same source as for the sulfate-reducing enrichments. Mixed sediment and groundwater (~2% v/v) was used to initiate five ethanol-fed iron-reducing enrichments. Fe(III) was provided in the form of amorphous ferric hydroxide. The growth medium is