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Bioreduction of U(VI) and stability of immobilized uranium under suboxic conditions

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ABSTRACT

In order to study the bioreduction of U(VI) and stability of immobilized uranium under suboxic conditions, microcosm were amended with ethanol, lactate and glucose, and incubated under suboxic conditions. During the incubation, total dissolved U in amended microcosms decreased from 0.95 mg/L to 0.03 mg/L. Pyrosequencing results showed that, the proportion of anaerobic microorganisms capable of reducing U(VI) under suboxic conditions was small compared with that under anoxic conditions; the proportion of aerobic and facultative anaerobic microorganisms capable of consuming the dissolved oxygen was large; and some of the facultative anaerobic microorganisms could reduce U(VI). These results indicated that different microbial communities were responsible for the bioreduction of U(VI) under suboxic and anoxic conditions. After the electron donors were exhausted, total dissolved U in the amended microcosms remained unchanged, while the U(VI)/U(IV) ratio in the solid phase of sediments increased obviously. This implied that the performance of bioreduction of the U(VI) can be maintained under suboxic condition.

1. Introduction

Groundwater contamination by uranium is a widespread problem at uranium tailings impoundments across the world (Neves and Matias, 2008). Uranium in the groundwater poses a threat to the health of the residents and the ecological environment around the uranium tailings impoundments. Reductive biostimulation is a promising bioremediation strategy for uranium contaminated groundwater, and this approach reduces soluble U(VI) to sparingly soluble U(IV) and greatly decreases the mobility of uranium in groundwater (Yabusaki et al., 2007).

The indigenous microbial communities in groundwater have been found to be stimulated by various electron donors, and certain microbial communities such as the *Geobacter* species, *Desulfotomaculum* species, and *Shewanella* species etc. have been found to be capable of reducing U(VI) (Lovley et al., 1991, 1993; Lovley and Phillips, 1992). Sulfate reducing bacteria (SRB) and iron reducing bacteria (IRB) have been found to be favorable for bioreduction of U(VI) and the stability of the biogenic U(IV) (Cardenas et al., 2010; Mohanty et al., 2008; Boonchayaanant et al., 2009). Iron hydroxide may compete with uranyl as a terminal electron acceptor and thereby retard biological uranium reduction and precipitation by dissimilatory metal-reducing bacteria (Wielinga et al., 2000). Uranium could be adsorbed and retarded on the Fe-oxide minerals in the sediments (Duff et al., 2002). The high concentration sulfate radica released from the sulfate minerals have been found to be unfavorable for the U(VI) reduction and U(IV)/U(VI) sorption (Spear et al., 2000; Frost et al., 2005). Therefore, the influence of the stimulated microbial communities in groundwater under suboxic conditions on the bioreduction of U(VI) needs to be studied thoroughly.

The ubiquitous dissolved oxygen (DO) in groundwater is an important factor influencing bioreduction of U(VI) since it can reoxidize the biogenic U(IV), and the DO can affect the microbial communities during the bioreduction of U(VI) (Komlos et al., 2008; Moon et al., 2009). N'Guessa found that U(VI) was reduced in groundwater containing low level DO after acetate was added, and the reoxidation of the biogenic U(IV) was greatly retarded after the termination of acetate addition (N'Guessan et al., 2010). Wu et al. found that sulfate and Fe(III) reduction occurred when ethanol and

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DO were present in groundwater, and the bioreduction of U(VI) was observed; uranium concentration increased significantly when ethanol was absent and DO was present (Wu et al., 2007). Campbell et al. found that U(IV) reoxidation rates were retarded by the biomass, cell exudates, and the geochemical conditions of groundwater containing low oxygen (Campbell et al., 2011). Therefore, studies on the bioreduction of U(VI) and the stability of the biogenic U(IV) under suboxic conditions are important because groundwater in many sites are under suboxic conditions.

The objective of the present research was to study the microbial communities assisting in U(VI) bioreduction and the biogeochemical factors influencing the stability of the biogenic U(IV) under suboxic conditions. In order to accomplish this, microcosms were prepared and amended with ethanol, lactate and glucose, and incubated under suboxic conditions. The variation of pH, chemical oxygen demand (COD), nitrate, sulfate, uranium and DO were monitored during the incubation. The 16s rRNA pyrosequencing was used to analyze the microbial communities assisting in the reduction of U(VI), and the X-ray absorption near edge structure (XANES) analysis and chemical extraction were used to determine the proportion between U(VI) and U(IV) in solid phase of sediments.

2. Materials and methods

2.1. Sampling site

The sediment and groundwater samples were taken from the uranium contaminated groundwater near a decommissioned uranium tailings impoundment in South China, and its hydrogeological characteristics were described in previous literature (Nie et al., 2010). The impoundment was covered with uncontaminated clay, and the monitoring wells for the groundwater were drilled outside the impoundment after its decommissioning. The residual uranium in the tailings was leached into the wastewater within the impoundment, and uranium was detected in the groundwater. The concentration of the DO in groundwater was between 0.2 and 0.5 mg/L from its bottom to its top. The concentration of total dissolved U in groundwater sampled at depth of 20.5 m below the surface was 0.5 mg/L, and it was 2.0 mg/L at depth of 9.2 m. Both measurements were much higher than the China's maximum contaminant limit for uranium of 0.050 mg/L for drinking water (GB23727-2009) prescribed by the Standardization Technology Committee of the National Nuclear Industry. A seepage collecting system was constructed outside the dam of the impoundment, and the collected wastewater was pumped back to the impoundment. There is a river which is only 3.5 km away, and the uranium contaminated groundwater poses a threat to the river.

2.2. Sample collection

The groundwater samples were collected with a rope-tied sampling bottle at 18 m depth from a monitoring well which is 10 cm in diameter and 20 m in depth in the uranium tailing impoundments, and they were then put into the sterilized glass bottles in an anaerobic glove bag. The sediment samples were collected from the same monitoring well with a sediment collector, and they were then transferred into the sterilized anaerobic glass bottles and put into the anaerobic glove bag filled with N_2. Both the groundwater and the sediment samples were delivered to the laboratory immediately. Some of the groundwater samples were then filtered through a 0.2 μm filter and were to be used for chemical analysis. The unfiltered groundwater samples together with some of the sediment samples were anaerobically stored overnight at 4 °C for microcosm experiments, and other sediment samples were anaerobically stored at −20 °C for future analysis.

2.3. Suboxic incubation of microcosms

Firstly, 50 g sediment and 800 mL groundwater were put into each one of the four 1 L sterilized jars, one of 3.622 g glucose (AR), or 3.589 g lactate (AR), or 2.340 mL ethanol (AR, 99.5%, w/w) were added into three of the four jars, making the equivalent COD in each jar be 4.8 g/L. Afterwards, 2.000 g sodium bicarbonate (AR) was added into each jar, making the bicarbonate concentration in it be 2.5 g/L. The three amended jars were used for the experiment, and the unamended jar was used for the control. Then, all jars were bubbled with N_2/CO_2 (4:1) gases for 30 min to remove the DO in the solution, and were put into the glove box to which two gas filters were connected. Finally, the glove box was evacuated to −0.05 MP, a gas mixture of 1.2% O_2 and 98.8% N_2 was injected into the box, the proportion between O_2 and N_2 remained unchanged so as to make the DO concentration in the control be 0.5 mg/L, and the jars were incubated statically in the dark at room temperature (Campbell et al., 2011). All the treatments were performed in triplicate. After the incubation began, samples were taken from the jars nearly once a week, and the DO was monitored every 2 days.

2.4. Analysis of sediment and water samples

Moisture content of a sediment sample was determined by weighing it before and after drying at 60 °C for 24 h. All water samples were filtered through a 0.2 μm filter and were stored at 4 °C until they were analyzed. The sediment samples for microbial community analysis were taken at day 60 and day 90 and were stored at −20 °C. Metal elements in the sediment and groundwater samples were analyzed using the atomic absorption spectrometry after acid digestion ($HF/HClO_4/HNO_3$) (PerkinElmer, PinAAcle 900E, USA). The pH was measured with pH Meter (PHS-25, INESA Scientific Inc., China). Sulfate, nitrate, and COD were determined with spectrophotometry (T6 series UV visible spectrophotometer, Purkinje General, China) (SEPA, 2002). The DO was measured with Clarke-type oxygen microelectrodes with a guard cathode (Unisense AS, Aarhus, Denmark). Bioavailable Fe(III) was quantified with a 1 h HCl extraction followed by ferrozine analysis (Lainie et al., 2003). Aqueous total dissolved U was analyzed using ICP-MS (Agilent, 7700 X, USA) with a detection limit for uranium of 0.001 mg/L.

2.5. Chemical extraction of U(VI) and U(IV)

In order to determine the proportions of the adsorbed U(VI) and the biogenic U(IV) at different times, sediment samples of about 0.5 g were first extracted anaerobically for 48 h in 5 mL of 100 mM sodium bicarbonate solution (pH 8.4), and then the sediment-bicarbonate slurry was kept in the air for 24 h to oxidize U(IV) to U(VI) (N'Guessan et al., 2008; Senko et al., 2002). In this way, the total uranium, adsorbed U(VI) and biogenic U(IV) in the sediment can be determined.

2.6. U L_3-edge XANES measurements

The fluorescence U L3-edge (17,166 eV) XAFS measurements were performed at beam line BL14W1 at Shanghai Synchrotron Radiation Facility, China. All the spectra were collected in fluorescent mode at room temperature. The fluorescent X-rays were all monitored with an ionization chamber filled with a 1:1 mixture of N_2 and He gases. The station used a Si(111) double crystal monochromator to select energy. During the measurement, the synchrotron was operated at energy 3.5 GeV. The photon energy was