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Interactions of uranium with bacteria and kaolinite clay

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Abstract

We assessed the accumulation of uranium (VI) by a bacterium, *Bacillus subtilis*, suspended in a slurry of kaolinite clay, to elucidate the role of microbes on the mobility of U(VI). Various mixtures of bacteria and the koalinite were exposed to solutions of 8×10^{-6} M- and 4×10^{-4} M-U(VI) in 0.01 M NaCl at pH 4.7. After 48 h, the mixtures were separated from the solutions by centrifugation, and treated with a 1 M CH_3COOK for 24 h to determine the associations of U within the mixture. The mixture exposed to 4×10^{-4} M U was analyzed by transmission electron microscope (TEM) equipped with EDS. The accumulation of U by the mixture increased with an increase in the amount of *B. subtilis* cells present at both U concentrations. Treatment of kaolinite with CH_3COOK, removed approximately 80% of the associated uranium. However, in the presence of *B. subtilis* the amount of U removed was much less. TEM–EDS analysis confirmed that most of the U removed from solution was associated with *B. subtilis*. XANES analysis of the oxidation state of uranium associated with *B. subtilis*, kaolinite, and with the mixture containing both revealed that it was present as U(VI). These results suggest that the bacteria have a higher affinity for U than the kaolinite clay mineral under the experimental conditions tested, and that they can immobilize significant amount of uranium.

Keywords: Uranium; *Bacillus subtilis*; Kaolinite; Accumulation; Bacteria–mineral mixture; Migration

1. Introduction

The migration of uranium from uranium-mining operations and the disposal of radioactive wastes are major environmental concerns (Buck et al., 1996; Airey and Ivanovich, 1986). Uranium typically occurs

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as hexavalent uranyl aqueous complexes in oxic environments (Langmuir, 1978). The mobility of U(VI) is determined by its interactions with soils and subsoils composed of abiotic and biotic components, principally minerals and bacteria, respectively (Dent et al., 1992; Ticknor, 1994; Waite et al., 1994; Sylwester et al., 2000; Fowle et al., 2000; Haas et al., 2001; Francis et al., 2004). There have been extensive studies done on the accumulation of U(VI) by bacteria (Lovley et al., 1991; Fowle et al., 2000; Haas et al., 2001; Brantley et al., 2001; Francis et al., 2004) and by minerals (Dent et al., 1992; Ticknor, 1994; Waite et al., 1994; Sylwester et al., 2000). However, as far as we are aware, little is known about U sorption in a mixture of bacteria and minerals.

Studies of U(VI) interactions with bacteria showed that U(VI) may be associated with the functional groups on the cellular surface (Fowle et al., 2000; Haas et al., 2001; Francis et al., 2004), precipitated to form uranyl-containing minerals (Macaskie et al., 1992; Young and Macaskie, 1995; Jeong et al., 1997), or reduced to insoluble U(IV) (Lovley et al., 1991; Francis et al., 1994; Suzuki et al., 2002). Studies with alumino-silicates minerals revealed that U(VI) is absorbed by the reactive groups of the minerals at pHs between 3 and 5. Therefore, an understanding of the behavior of U(VI) in soils and rocks requires a detailed knowledge of its interactions not only with the bacterial and mineral surfaces, but also within a mixture of bacteria and minerals.

In this study we investigated the accumulation of U by mixtures of *Bacillus subtilis* and kaolinite. *B. subtilis* and kaolinite were chosen because (a) both are ubiquitous in the terrestrial environment; and, (b) their surfaces are well characterized. We also determined whether U showed preferential affinity to bacteria, kaolinite clay or in a mixture of both. In addition to carrying out sorption and desorption experiments, we examined the association of U by transmission electron microscopy (TEM).

2. Materials and methods

2.1. Microorganism, kaolinite, and U solution

B. subtilis (IAM 1069) was obtained from the Institute of Molecular and Cellular Biosciences, The University of Tokyo. This strain is a Gram-positive, rod-shaped heterotrophic bacterium. The cells were grown for 40–48 h in 500-mL conical flasks at 30 °C in 250 mL sterilized liquid growth medium containing meat extract (3 g L^{-1}), polypeptone (5 g L^{-1}), and NaCl (5 g L^{-1}). Cells at the stationary growth phase were harvested by centrifugation at 2500×*g* for 10 min, and washed twice by 0.1 M NaCl. They finally were resuspended in a 0.01 M NaCl solution and immediately used in the experiments. An aliquot of the cell suspension was centrifuged and dried overnight at 70 °C to determine the dry weight of the cells in the stock suspension.

Commercial kaolinite from Nihon Chikagaku-sha Co. Ltd., Kyoto, Japan was used. Kaolinite was the only mineral identified by X-ray powder diffraction. The specific surface area of the kaolinite was 26.4 m^2 g^{-1}, assessed by the Brunaure Emmett Teller (BET) method. The kaolinite was washed twice with distilled deionized water and suspended as a slurry to a concentration of 100 g L^{-1}.

Natural U stock solutions of different U concentrations were prepared by dissolving $UO_2(NO_3)_2 \cdot 6H_2O$ in 0.01 M NaCl. The ^{233}U isotope was added to the natural U stock solutions to achieve the activity required for radiometric measurements.

2.2. Accumulation experiments

The accumulation of U(VI) by biotic and/or abiotic components depends on the chemical species of U(VI). At pHs between 3 and 5, U(VI) is present predominantly as dissolved $U(VI)O_2^{2+}$ species (Suzuki and Banfield, 1999). In solution above pH 7, its major chemical species are uranyl carbonate complexes; a small portion of U(VI) can be precipitated to form uranyl hydroxides (Langmuir, 1978). We choose a solution at pH 4.7 to avoid the precipitation of U(VI) hydroxide and formation of U(VI) carbonate complex species.

To explore the accumulation of U(VI) in mixtures of *B. subtilis* and kaolinite, we exposed mixtures containing 38 g L^{-1} kaolinite and 0, 0.19 (0.5% in dry weight percent fraction), 0.38 (1%), 2.0 (5%), or 4.2 g L^{-1} (10%) of *B. subtilis* to an 8×10^{-6} M U(VI) solution at pH 4.7 ± 0.1 for 48 h . The mixtures containing 38 g L^{-1} kaolinite and 0, 0.04 (0.1%), 0.19 (0.5%), 0.38 (1%), 1.2 (3%), or 2.0 g L^{-1} (5%)