Biological Immobilization of Dissolved Uranium - 11578

Seung Yeop Lee and Min Hoon Baik

Korea Atomic Energy Research Institute, 1045 Daedeok-daero, Yuseong-gu, Daejeon,

Korea

ABSTRACT

Biogenic UO_2 (uraninite) nanocrystals may be formed as a product of a microbial reduction process in uranium-enriched environments near the Earth's surface. We investigated the size, nanometer-scale structure, and aggregation state of UO_2 formed by iron-reducing bacterium, *Shewanella putrefaciens* CN32, from a uranium-rich solution. Characterization of biogenic UO_2 precipitates by high-resolution transmission electron microscopy (HRTEM) revealed that the UO_2 nanoparticles formed were highly aggregated by organic polymers. Nearly all of the nanocrystals were networked in more or less 100 nm diameter spherical aggregates that displayed some concentric UO_2 accumulation with heterogeneity. When phosphate was added to the system, calcium was found to be easily associated with uranium(IV), forming a new uranium phase, ningyoite. These results will extend the limited knowledge of microbial uraniferous mineralization and may provide new insights into the fate of aqueous uranium complexes.

INTRODUCTION

Dissimilatory metal-reducing bacteria (DMRB) can couple the oxidation of organic matter or H_2 to the reduction of oxidized radionuclides. Usually, oxidized uranium(VI) is much more soluble than the reduced form, uranium(IV), and typically exists in groundwater as uranyl carbonate complexes [1-3]. Oxidized uranium is readily reduced by DMRB under anoxic conditions, resulting in the precipitation of UO_2 nanoparticles [4, 5]. The rapid rate of oxidized uranium reduction and the low solubility of the reduced form make bioremediation an attractive option for removing uranium from contaminated groundwaters [6-9].

Biogenic UO_2 is a fascinating and important nanoscale biogeological material. The long-term structural stability of biogenic UO_2 is crucial to the viability of microbial bioremediation strategies [10] that seek to mitigate subsurface uranium contamination. For example, Hanford site in the United States is a typical area contaminated with uranium, and the uranium which is mobile with groundwater is needed to be immobilized in situ by indigenous bacteria. UO_2 nanoparticles are potentially highly mobile because of their small size and can redissolve quickly if conditions change [11, 12]. Size, shape, structure, degree of crystallinity, and polymer associations all affect UO_2 solubility, transport in growndwater, and potential for deposition by sedimentation.

The fate of uranium in natural systems is of great environmental importance. Because a long-term behavior of uranium can cause unpredictable results on natural ecosystem.

In addition, in a high-level radioactive waste repository, uranium migration should be estimated for the purpose of the ecological safety of the region.

We report the detailed structure of aggregated nanobiogenic uraninite produced by *Shewanella putrefaciens* CN32 in the presence of major cations of groundwater. Some uranium ore deposits are believed to involve a direct microbial reduction process for uranium(VI) [4, 13], as opposed to an abiotic reduction by reduced species such as sulfide [14], magnetite [15], and green rust [16]. We document the aggregation of nanoparticles to form submicrometer-scale aggregates by organic polymers, and crystal growth pathways that can lead to morphologies similar to those found in sedimentary environments.

EXPERIMENTAL

S. putrefaciens strain CN32 (ATCC BAA-1097) was obtained from the American Type Culture Collection (ATCC), USA. The *S. putrefaciens* CN32 was routinely cultured aerobically in a 30 g/L tryptic soy broth (TSB) (Difco Laboratories, Detroit, MI, USA), and stock cultures were maintained by freezing them in 40% glycerol at -80°C.

The aerobically cultured *S. putrefaciens* CN32 cells were harvested at mid to late log phase by centrifugating them from 30 g/L TSB cultures. The cells were centrifuged at 4,000 rpm for 15 min. The supernatant was discarded and the cell pellets were suspended in a 30 mmol/L $NaHCO_3$ (pH 7) buffer solution and purged with N_2 gas. This process was repeated four times and washed cells ($>4\times10^8$ cells mL^{-1}) were used as inoculum.

The $NaHCO_3$ buffer solution (30 mM) was extensively flushed with N_2 to remove

3

dissolved O_2. 100 mL of the buffer solution with lactate (10 mM) as an electron donor were dispensed into 120 mL serum bottles under N_2 condition. The headspace of the serum bottles was pressurized with ultrapure nitrogen, then capped with butyl rubber septa and crimped with an aluminum seal. The bottle and solution were sterilized by autoclaving at 121°C for 20 min.

For simulating the natural electrolytic content of natural groundwater, several filtered (0.2 µm, Advantec cellulose acetate) stock solutions of major cations were aseptically added by syringe and needle to the serum bottles as soluble forms as follows (mM): calcium chloride, 1.0; potassium chloride, 1.0; magnesium chloride, 1.0. In addition, P was separately injected into some of those bottles as a form of sodium hydrogen phosphate (0.3 mM).

Uranium(VI) stock solutions were prepared by dissolving a known amount of $UO_2(NO_3)_2 \cdot 6H_2O$ (Aldrich) in a previously acidified $HClO_4$ solution to prevent cation hydrolysis. The stock solution concentrations were about 1×10^{-3} M, and uranium(VI) (5×10^{-5} M) was aseptically added using a purged needle and syringe. Finally, washed *S. putrefaciens* CN32 cells were injected into the serum bottles to create a final concentration of 8 mg/L cell protein. The final pH of the solution of the serum bottle was ~8.0. The solution pH was measured using a Ross combination pH electrode and an Orion 920A pH/ISE/mV/EC meter. The inoculated serum bottles were then put into a rotary-shaker (120 rpm at 25°C) in the dark. Periodically, 2-mL samples were aseptically removed by syringe and needle through 0.2 µm cellulose acetate filters and were analyzed for soluble concentrations of major ions, including uranium, using an inductively coupled plasma mass

spectrometry (ICP/MS).

For the electron microscopic observation, the precipitate residue of the centrifuged suspension was washed twice with N_2-purged water. A high-resolution transmission electron microscopy (HRTEM) analysis was performed with samples collected from the experiment to observe bacterial surfaces and newly-formed nanomaterials. In order to see the bacterial surface in detail without disturbance, unstained samples were prepared by drying diluted aliquots of suspension on holey carbon films that were coated with a very thin layer of amorphous carbon (~5-10 nm thickness) on copper and formvar-mesh HRTEM grids. The prepared samples were immediately observed using HRTEM (JEOL JEM 2100F) at 200 kV. EDS analysis was also used to detect newly-formed nanoparticles and analyze the distribution of major elements.

RESULTS and DISCUSSION

During microbial respiration, the initial aqueous uranium concentration (5×10^{-5} M) continued to slowly decrease to a very low level for about 2 weeks (Fig. 1). The continuous decline of aqueous uranium was derived from the bioreduction of uranium(VI) to uranium(IV), which is less soluble and generally tends to precipitate as UO_2.
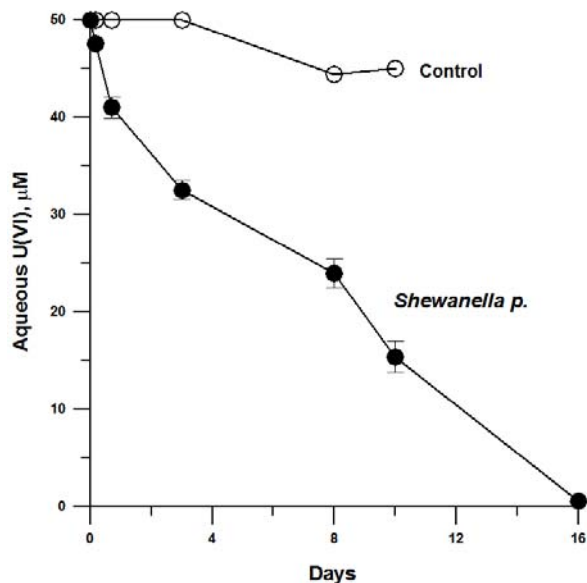
Fig. 1. Uranium(VI) reduction by *S. putrefaciens* strain CN32 in 30 mmol/L, pH 8.1 NaHCO$_3$ buffer. A 'Control' sample has no bacterium.

The UO$_2$ formed by the iron-reducing bacterium, *S. putrefaciens* CN32, was characterized by HRTEM (Fig. 2). Microbial UO$_2$ nanoparticles were highly aggregated by organic polymers. The detected uranium appeared as aggregate balls measuring 50-100 nm in diameter. Observed aggregates of >100 nm in diameter may have been formed as smaller ones were closely linked by organic ligands. The interlinking of aggregates usually occurred in cases of short distance. Through such a process, the enlarged-aggregate was likely to organize organic-based UO$_2$ networks, serving as stable bases for catching mobile small-sized UO$_2$. Some adsorbed uranium particles were present as locally concentric over the aggregates. The much brighter parts of the aggregates indicate some specific uranium-rich places, indicating that pure uranium particles were not accumulated homogeneously but locally.
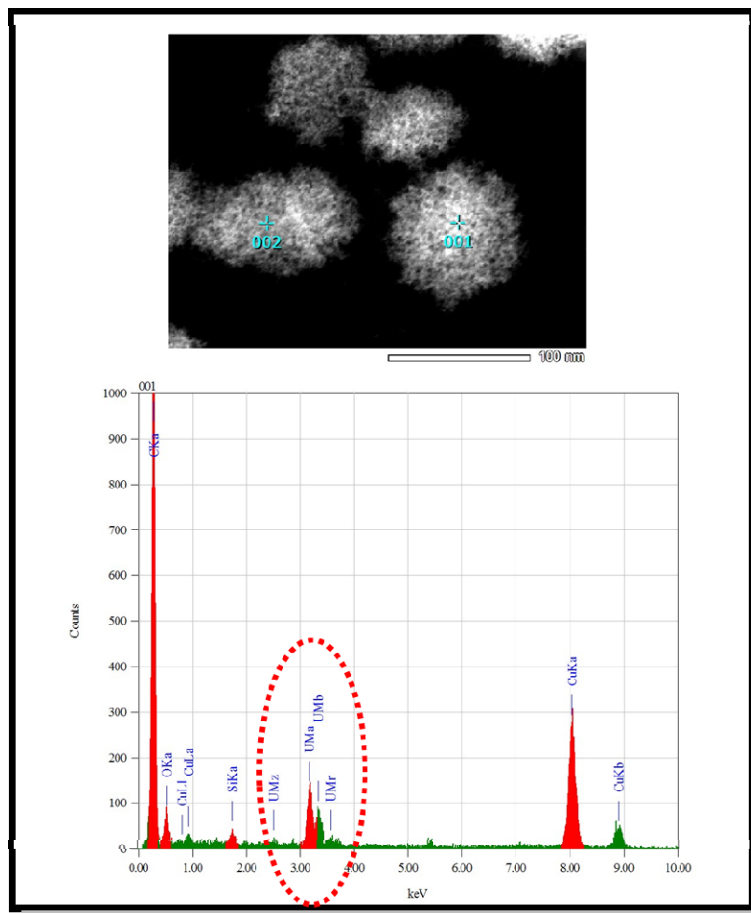
Fig. 2. A HRTEM image of aggregated UO_2 nanocrystals and a EDS spectrum. Nanoparticles are aggregated with organic polymers in more than 100 nm size and have uranium and carbon components. A dotted circle in the EDS spectrum shows uranium peaks for two spots (001 and 002) in the upper image. Cu peaks were derived from the TEM grid.

Some researchers reported that c-type cytochromes of DMRB are essential for the reduction of U(VI) and the formation of extracelluar UO_2 nanoparticles [17, 18]. In particular, the outer membrane (OM) decaheme cytochrome MtrC (metal reduction) has

7

been known to directly transfer electrons to U(VI) [17]. Through this process, uranium(VI) is bioreduced to uranium(IV), and the next step involves the precipitation of the biogenic uranium phase. Microbial organic molecules may induce a rapid capture of the uranium phase. Microbial polymers are known to scavenge nanoparticles [19, 20]. Microbially-derived extracellular polymeric substance (EPS) could limit the dispersal of the nanoparticulate uranium-bearing phase that may otherwise be transported away from its source by subsurface fluid flow.

When phosphate was loaded in the system, a new solid phase was formed by the same bacterium, *S. putrefaciens* CN32. Fig. 3 shows the bacterium encrusted with nanometer-size crystallites, which were identified as ningyoite, a uranium calcium phosphate mineral. This spheroidal morphology accords well with that of the microbially synthesized ningyoite ($CaU(PO_4)_2 \cdot H_2O$) by Khijniak et al. [21].

Incorporation of groundwater-dissolved cations into the uranium phase will be critical to predicting uranium-bearing nanoparticle stability and growth in the environment. Most equilibrium speciation models predict that the dominant uranium aqueous species in groundwater will be uranyl carbonate complexes [22, 23]. Generally, Ca-U-CO_3 complexes ($CaUO_2(CO_3)_3^{2-}$, $Ca_2UO_2(CO_3)_3$) have been proposed to play an important role in the environmental chemistry of uranium [8]. Calcium is usually a common ion in groundwater that can easily complex with uranium(VI) in bicarbonate solutions.
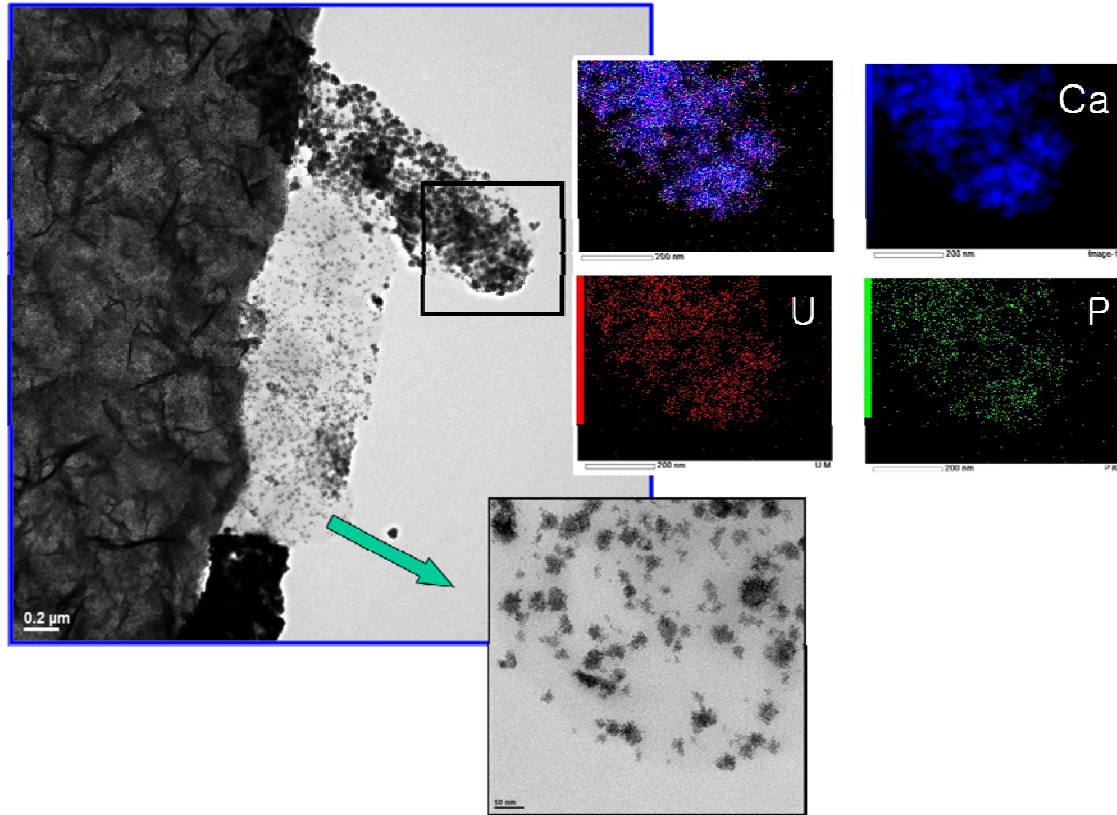
Fig. 3. HRTEM images of *S. putrefaciens* CN32 showing uranium phosphate particles precipitated on the cell surfaces, and an enlarged view of aggregated particles and elemental distributions.

Unfortunately, for the above reasons, the bioreduction rate of aqueous uranium(VI) with calcium was slower than that of uranium(VI) without calcium under the same cell concentration [1]. The calcium caused a significant decrease in the rate and extent of bacterial uranium(VI) reduction [2, 3, 8]. Interestingly, in our study when uranium(VI) with calcium was reduced to uranium(IV), the calcium did not consistently combine with uranium(IV) in the formation of a uranium phase. The calcium appears to be neglected from the UO_2 nucleation process. This means that calcium is no longer complexed with

uranium(IV) when uranium(VI) is bioreduced to uranium(IV), probably due to the lower binding energy of calcium for uranium(IV).

In spite of the above result, when phosphate was added to the system, calcium was found to be easily associated with uranium(IV) forming a new uranium phase (ningyoite).

$$\underline{CaUO_2(CO_3)_3}^{2-} + 2Na_2H(PO_4) + 5H^+ \rightarrow \underline{CaU(PO_4)_2 \cdot 2H_2O} + 3HCO_3^- + 4Na^+$$

(uranium complex)   (ningyoite)

The intimate relationship between uranium and calcium appears to be maintained by phosphate, even though uranium(VI) was changed to uranium(IV) during microbial reduction. We suppose that the separation of calcium from uranium(IV) is retarded by the adhesion of phosphate, which can promote their coprecipitation regardless of the uranium oxidation state. However, if aqueous uranium(VI) or phosphate concentrations were elevated to mmole/L level, an abiotic precipitation of uranium(VI) phosphate phases could occur due to their rapid coprecipitation [21] without affordable bioreduction. Nevertheless, a microbial reduction (bio-transformation) for the solid-phase U(VI) to U(IV) can occur, but it may need much more time and energies [21, 24]. As amendments of backfill material for radioactive waste storage, phosphate is currently considered one of the most important candidate chemicals [25]. Considering this situation, the microbial phosphatic uranium(IV) phase, due to its extremely low solubility under circumneutral pH conditions [21], might play an important role in lowering uranium mobility in uranium-rich environments.

CONCLUSION

Biogenic UO_2 precipitates produced by DMRB were observed to be aggregated to several hundred nm sizes with particle-oriented attachment. The aggregation of UO_2 nanoparticles implies a growth of UO_2. This phenomenon explains a natural origin of biogenic uraninite, "pitchblende". The UO_2 growth may be a very important factor in a bioremediation strategy for uranium-contaminated sites to immobilize and stabilize dissolved uranium in situ. Furthermore, a new insoluble uranium phase, ningyoite, can be also a useful material product to restrain the migration of uranium in uranium-rich sites.

REFERENCES

1. C. Liu, B.H. Jeon, J.M. Zachara, and Z. Wang, "Influence of calcium on microbial reduction of solid phase uranium(VI)", Biotech. Bioeng. 97, 1415-1422 (2007).

2. J. Neiss, B.D. Stewart, P.S. Nico, and S. Fendorf, "Speciation-dependent microbial reduction of uranium within iron-coated sands", Environ. Sci. Technol. 41, 7343-7348 (2007).

3. B.D. Stewart, J. Neiss, and S. Fendorf, "Quantifying constraints imposed by calcium and iron on bacterial reduction of uranium(VI)", J. Environ. Qual. 36, 363-372 (2007).

4. D.R. Lovley, E.J.P. Phillips, Y.A. Gorby, and E.R. Landa, "Microbial reduction of uranium", Nature 350, 413-416 (1991).

5. Y.A. Gorby, and D.R. Lovley, "Enzymatic uranium precipitation", Environ. Sci. Technol. 26, 205-207 (1992).

6. Y. Suzuki, S.D. Kelly, K.M. Kemner, and J.F. Banfield, "Nanometre-size products of uranium bioreduction", Nature 419, 134 (2002).

7. J.K. Fredrickson, J.M. Zachara, D.W. Kennedy, C. Liu, M.C. Duff, D.B. Hunter, and A. Dohnalkova, "Influence of Mn oxides on the reduction of uranium(VI) by the metal-reducing bacterium *Shewanella putrefaciens",* Geochim. Cosmochim. Acta 66, 3247-3262 (2002).

8. S.C. Brooks, J.K. Fredrickson, S.L. Carroll, D.W. Kennedy, J.M. Zachara, A.E. Plymale, S.D. Kelly, K.M. Kemner, and S. Fendorf, "Inhibition of Bacterial U(VI) reduction by calcium", Environ. Sci. Technol. 37, 1850-1858 (2003).

9. W.D. Burgos, J.T. McDonough, J.M. Senko, G. Zhang, A.C. Dohnalkova, S.D. Kelly, Y. Gorby, and K.M. Kemner, "Characterization of uraninite nanoparticles produced by *Shewanella oneidensis* MR-1", Geochim. Cosmochim. Acta 72, 4901-4915 (2008).

10. B. Wielinga, B. Bostick, C.M. Hansel, R.F. Rosenzweig, and S. Fendorf, "Inhibition of bacterially promoted uranium reduction: Ferric (hydr)oxides as competitive electron acceptors", Environ. Sci. Technol. 34, 2190-2195 (2000).

11. K.A. Ulrich, A. Singh, E.J. Schofield, J.R. Bargar, H. Veeramani, J.O. Sharp, R. Bernier-Latmani, and D.E. Giammar, "Dissolution of biogenic and synthetic UO_2 under varied reducing conditions", Environ. Sci. Technol. 42, 5600-5606 (2008).

12. K.A. Ulrich, E.S. Ilton, H. Veeramani, J.O. Sharp, R. Bernier-Latmani, E.J. Schofield, J.R. Bargar, and D.E. Giammar, "Comparative dissolution kinetics of biogenic and chemogenic uraninite under oxidizing conditions in the presence of carbonate", Geochim. Cosmochim. Acta 73, 6065-6083 (2009).

13. A. Mohagheghi, D.E. Updegraff, and M.B. Goldhaber, "The role of sulfate-reducing bacteria in the deposition of sedimentary uranium ores", Geomicrobiol. J. 4, 153-173 (1985).

14. B. Hua, H.F. Xu, J. Terry, and B.L. Deng, "Kinetics of uranium(VI) reduction by hydrogen sulfide in anoxic aqueous systems", Environ. Sci. Technol. 40, 4666-4671 (2006).

15. T.B. Scott, G.C. Allen, P.J. Heard, and M.G. Randell, "Reduction of U(VI) to U(IV) on the surface of magnetite", Geochim. Cosmochim. Acta 69, 5639-5646 (2005).

16. E.J. O'Loughlin, S.D. Kelly, R.E. Cook, R. Csencsits, and K.M. Kemner, "Reduction of

uranium(VI) by mixed iron(II)/iron(III) hydroxide (green rust): Formation of UO_2 nanoparticles", Environ. Sci. Technol. 37, 721-727 (2003).

17. M.J. Marshall, A.S. Beliaev, A.C. Dohnalkova, D.W. Kennedy, L. Shi, Z. Wang, M.I. Boyanov, B. Lai, K.M. Kemner, J.S. McLean, S.B. Reed, D.E. Culley, V.L. Bailey, C.J. Simonson, D.A. Saffarini, M.F. Romine, J.M. Zachara, and J.K. Fredrickson, "c-Type cytochrome-dependent formation of U(IV) nanoparticles by Shewanella oneidensis", PLoS Biology 4, 1324-1333 (2006).

18. E.S. Shelobolina, M.V. Coppi, A.A. Korenevsky, L.N. DiDonato, S.A. Sullivan, H. Konishi, H. Xu, C. Leang, J.E. Butler, B.C. Kim, and D.R. Lovley, "Importance of c-Type cytochromes for U(VI) reduction by Geobacter sulfurreducens", BMC Microbiology 7, 16 (2007).

19. E.G. Sogaard, R. Medenwaldt, and J.V. Abraham-Peskir, "Conditions and rates of biotic and abiotic iron precipitation in selected Danish freshwater plants and microscopic analysis of precipitate morphology", Water Res. 34, 2675-2682 (2000).

20. D. Mavrocordatos, and D. Fortin, "Quantitative characterization of biotic iron oxides by analytical electron microscopy", Amer. Miner. 87, 940-946 (2002).

21. T.V. Khijniak, A.I. Slobodkin, V. Coker, J.C. Renshaw, F.R. Livens, E.A. Bonch-Osmolovskaya, N.K. Birkeland, N.N. Medvedeva-Lyalikova, and J.R. Lloyd, "Reduction of uranium(VI) phosphate during growth of the thermophilic bacterium *Thermoterrabacterium ferrireducens",* Appl. Environ. Microbiol. 71, 6423-6426 (2005).

22. D.L. Clark, D.E. Hobart, and M.P. Neu, "Actinide carbonte complexes and their importance in actinide environmental chemistry", Chem. Rev. 95, 25-48 (1995).

23. A. Abdelouas, Y. Lu, W. Lutze, and H.E. Nuttall, "Reduction of U(VI) to U(IV) by indigenous bacteria in contaminated ground water", J. Contam. Hydrol. 35, 217-233 (1998).

24. I. Ortiz-Bernad, R.T. Anderson, H.A. Vrionis, and D.R. Lovley, "Resistance of solid-phase U(VI) to microbial reduction during in situ bioremediation of uranium-contaminated groundwater", Appl. Environ. Microbiol. 70, 7558-7560 (2004).

25. E.H. Oelkers, and J.M. Montel, "Phosphates and nuclear waste storage", Elements 4, 113-116 (2008).