Biom mineralization of U(VI) Phosphate Promoted by Microbially-Mediated Phytate Hydrolysis in Contaminated Soils

Kathleen R. Salome¹, Melanie J. Beazley^{2,4},
Samuel M. Webb³, Patricia A. Sobecky², Martial Taillefert^{1*}

¹ *School of Earth & Atmospheric Sciences, Georgia Institute of Technology, Atlanta, GA 30332*

² *Department of Biological Sciences, University of Alabama, Tuscaloosa, AL 35487*

³ *Stanford Synchrotron Radiation Lightsource, Menlo Park, CA 94025*

⁴ *Department of Chemistry, University of Central Florida, Orlando, FL 32816*

** Corresponding author phone: (404)-894-6043; e-mail: mtaillef@eas.gatech.edu*

Submitted to *Geochimica et Cosmochimica Acta*

8/3/16

1 **ABSTRACT**

2 The bioreduction of uranium may immobilize a significant fraction of this toxic
3 contaminant in reduced environments at circumneutral pH. In oxic and low pH environments,
4 however, the low solubility of U(VI)-phosphate minerals also makes them good candidates for
5 the immobilization of U(VI) in the solid phase. As inorganic phosphate is generally scarce in
6 soils, the biomineralization of U(VI)-phosphate minerals via microbially-mediated
7 organophosphate hydrolysis may represent the main immobilization process of uranium in these
8 environments. In this study, contaminated sediments were incubated aerobically in two pH
9 conditions to examine whether phytate, a naturally-occurring and abundant organophosphate in
10 soils, could represent a potential phosphorous source to promote U(VI)-phosphate
11 biomineralization by natural microbial communities. While phytate hydrolysis was not evident
12 at pH 7.0, nearly complete hydrolysis was observed both with and without electron donor at pH
13 5.5, suggesting indigenous microorganisms express acidic phytases in these sediments. While
14 the rate of hydrolysis of phytate generally increased in the presence of uranium, the net rate of
15 inorganic phosphate production in solution was decreased and inositol phosphate intermediates
16 were generated in contrast to similar incubations conducted without uranium. These findings
17 suggest uranium stress enhanced the phytate-metabolism of the microbial community, while
18 simultaneously inhibiting phosphatase production and/or activity by the indigenous population.
19 Finally, phytate hydrolysis drastically decreased uranium solubility, likely due to formation of
20 ternary sorption complexes, U(VI)-phytate precipitates, and U(VI)-phosphate minerals. Overall,
21 the results of this study provide evidence for the ability of natural microbial communities to
22 liberate phosphate from phytate in acidic sediments, possibly as a detoxification mechanism, and
23 demonstrate the potential utility of phytate-promoted uranium immobilization in subsurface

- 1 environments. These processes should be investigated in more detail with pure cultures isolated
- 2 from these sediments.

1. INTRODUCTION

Since the end of the Cold War Era, the United States Department of Energy (DOE) has shifted its focus from nuclear weapons proliferation to remediation of radionuclide and heavy metal contamination at former weapons development sites (DOE, 1997). The large number of DOE-managed facilities with a wide range of subsurface geochemical conditions, ranging from highly acidic (Oak Ridge, TN) to alkaline pH (Hanford, WA), makes developing a prescriptive bioremediation strategy appropriate for all subsurface conditions complicated (DOE, 1997). In particular, the presence of acidic environments, where metal reduction is unfavorable, commands the development of creative *in situ* remediation solutions for environmental uranium contamination (Istok et al., 2004; Kelly et al., 2008; North et al., 2004; Sitte et al., 2010; Van Nostrand et al., 2011; Wu et al., 2006a; Wu et al., 2006b).

The mobility of uranium in the subsurface is controlled by a combination of surface complexation reactions, precipitation reactions, and redox processes (Finch and Murakami, 1999; Hsi and Langmuir, 1985; Langmuir, 1997). Uranium exists primarily as U(IV) or U(VI) depending on the prevailing environmental redox conditions. In oxidizing conditions, U(VI) exists as the aqueous uranyl ion UO_2^{2+} and its hydroxyl complexes. Above pH 7.0, carbonates may enhance uranyl mobility by forming soluble uranyl-carbonate complexes and promoting the dissolution of U(IV) and U(VI) minerals (Langmuir, 1978, 1997). In addition, the presence of calcium at this pH may inhibit uranyl reduction or U(VI)-phosphate precipitation through formation of ternary calcium-uranyl-carbonate complexes (Brooks et al., 2003; Luo et al., 2007), though uranyl phosphate precipitation may prevail when phosphate concentrations are elevated (Salome et al., 2013). In contrast, below circumneutral pH, strong sorption to metal oxides (Han et al., 2007; Hsi and Langmuir, 1985; Plathe et al., 2013; Waite et al., 1994), clays (Bai et al.,

2009; Grabias et al., 2014; Meleshyn et al., 2009), and natural organic matter (NOM) (Li et al., 2014) and the formation of highly stable and sparingly soluble precipitates with phosphate may limit uranyl mobility in the environment (Finch and Murakami, 1999; Langmuir, 1997). In reducing conditions, U(VI) may either be chemically (Hua and Deng, 2008; Liger et al., 1999; Missana et al., 2003; O'Loughlin et al., 2003; Wersin et al., 1994) or microbially (Lovley et al., 1991; Sanford et al., 2007; Tebo and Obraztsova, 1998; Wade and DiChristina, 2000) reduced to U(IV) and precipitate as uraninite ($\text{UO}_{2(s)}$) (Finch and Murakami, 1999; Langmuir, 1997) or other non-uraninite U(IV) mineral phases (Alessi et al., 2014; Boyanov et al., 2011; Fletcher et al., 2010). However, the reintroduction of oxygen (Langmuir, 1997; Murphy and Shock, 1999; Wu et al., 2007) or the presence of NO_2^- (Senko et al., 2002; Senko et al., 2005a), Fe(III) (Senko et al., 2002; Senko et al., 2005b; Wan et al., 2005), or MnO_2 (Fredrickson et al., 2002) in reducing conditions may remobilize U(IV)-precipitates to soluble U(VI) species.

Thus far, *in situ* bioremediation of uranium contaminations has primarily focused on the formation of various reduced uranium mineral products via microbially-catalyzed reduction of U(VI), known as bioreduction (Bernier-Latmani et al., 2010; Istok et al., 2004; Kelly et al., 2008; Lovley et al., 1991; North et al., 2004; Sharp et al., 2011; Wade and DiChristina, 2000; Wu et al., 2006b). Unfortunately, the low pH, elevated nitrate, and oxidizing conditions present in some contaminated areas inhibit this process (Finneran et al., 2002; Wu et al., 2006a; Wu et al., 2006b), making the precipitation of U(VI)-phosphate minerals a potentially appealing alternative to bioreduction. As orthophosphate directly amended to the subsurface is rapidly immobilized thus limiting its transport (Wellman et al., 2006), research has focused on the addition of organophosphates to subsurface sediments to promote microbially-mediated organophosphate hydrolysis coupled to the chemical precipitation of sparingly soluble U(VI)-phosphate minerals

in a process termed biomineralization (Beazley et al., 2007, 2009; Beazley et al., 2011; Macaskie et al., 1994; Shelobolina et al., 2009). To fulfill their phosphorus requirement when faced with nutrient limitations, microorganisms encode genes for various phosphohydrolase (phosphatase) enzymes that catalyze the hydrolysis of organophosphates and release orthophosphate (Macaskie et al., 1994). In addition, uranium stress may induce microbial phosphatase expression as a metal detoxification mechanism (Barnett et al., 2002; Knox et al., 2008; Macaskie et al., 1994). Although previous research has demonstrated that microbial hydrolysis of synthetic organophosphates immobilizes more than 98% of U(VI) as U(VI)-phosphate minerals in both oxic (Beazley et al., 2007, 2009; Beazley et al., 2011; Martinez et al., 2007; Shelobolina et al., 2009) and suboxic (Beazley et al., 2009; Salome et al., 2013; Shelobolina et al., 2009) conditions, the potential of naturally-occurring organophosphates as a source of inorganic phosphorus to promote U(VI)-phosphate biomineralization has not been examined.

Inositol hexaphosphate (IP₆), also known as phytate, is a phosphorylated inositol with six attached phosphate groups (Figure S1) that is widely synthesized by plants for phosphorus storage and may represent the dominant organophosphate compound in terrestrial environments (Turner et al., 2002), with concentrations as high as 1 g P/kg soil (Turner, 2007). The accumulation of IP₆ in soils suggests that it is relatively unavailable for biological uptake or that its bioavailability depends on the presence of specific enzymes (Turner et al., 2002). Although IP₆ is chemically stable above pH 1 and is not hydrolyzed by conventional phosphohydrolases (Turner et al., 2002), phytase enzymes may catalyze its hydrolysis to lower inositol derivatives (IP_x, x = 1-5) and inorganic phosphate (Irving and Cosgrove, 1974). Phytase enzymes are substrate-specific phosphatases (Oh et al., 2004) that include both bacterial histidine acid phosphatases (HAP) (activated between pH 2 and 6) and bacterial β -propeller phytases (activated

at or above pH 7.0) (Oh et al., 2004). Acidic phytase enzymes are inhibited by divalent cat/ions (especially Ca^{2+}) (Oh et al., 2004), whereas alkaline phytase require Ca^{2+} for enzymatic function (Ha et al., 2000). Because of its highly negative charge above pH 2 (Figure 1A), phytate interacts strongly with soils (Degroot and Golterman, 1993; Johnson et al., 2012) and efficiently chelates metals to form sparingly soluble complexes (Martin and Evans, 1987; Turner et al., 2002). Although calcium phytate was demonstrated to chemically precipitate U(VI) by ion exchange (Knox et al., 2008; Nash et al., 1998; Seaman et al., 2003), the microbially-facilitated precipitation of U(VI)-phosphate minerals by phytate-mineralizing bacteria has yet to be examined. The objectives of this study were to investigate the ability of the natural microbial community in uranium-contaminated sediments of the ORFRC to metabolize phytate, examine the dependence of this metabolism on pH and the addition of an electron donor, and determine the geochemical conditions required to induce U(VI)-phosphate biomineralization coupled to microbial phytate degradation.

2. EXPERIMENTAL

All experiments were conducted in artificial groundwater, consisting of 15.4 mM NO_3^- , 0.81 mM SO_4^{2-} , 0.41 mM Cl^- , 7.5 mM Na^+ , 7.9 mM K^+ , Mg^{2+} 0.81 mM, Ca^{2+} 0.2 mM, Mn^{2+} 5.1 μM , and Fe^{2+} 2 μM buffered at either pH 5.5 or pH 7.0 with 50 mM 2-(N-Morpholino)ethanesulfonic acid (pH 5.5) or 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (pH 7.0).

2.1 Abiotic Precipitation of U(VI)-Phytate

To ensure phytate does not abiotically precipitate with uranium in the conditions of the incubations, triplicate solutions of 200 μM uranyl acetate (Spectrum) were equilibrated for 48 hours with 0, 0.05, 0.1, 0.2, 0.6, 1.0, 3.0, 5.0, and 10 mM Na-IP₆ (Sigma Aldrich) in artificial

groundwater buffered at either pH 5.5 or 7.0. After equilibration, a small aliquot was filtered through a 0.2 μm pore size polyethersulfone (PES) membrane filter (Puradisc, Whatman) and preserved in 2% trace metal grade nitric acid for uranium analysis.

2.2 Sediment Slurry Incubations

To examine the phytate metabolism of the natural microbial community, ORFRC Area 3 sediments (well number: FWB134-08-03, core depth: 6.5-7.5 m) were incubated in triplicate with artificial groundwater for 16 days under aerobic conditions. Area 3 sediments are generally characterized by low pH and high nitrate levels (Brooks, 2001). Although chemical information for well FWB134 is unavailable, the nearest neighboring wells (~8-12 m) display an average pH of 4.8 ± 1.5 , and Area 3 sediments may contain greater than 2 $\mu\text{mol/g}$ U (Gu et al., 2005), 500 $\mu\text{mol/g}$ Fe(III), and 1 mmol/g Al(III) hydroxides (Wu et al., 2006a). Sediments were stored in the dark at 4°C for approximately 3 months prior to incubation, and sediment cores remained sealed for the duration of their storage. In each treatment, homogenized sediment (4 grams) was combined with sterile artificial groundwater (250 mL) buffered at either pH 5.5 or 7.0 in sterilized 1 L erlenmeyer flasks. Incubation treatments included inositol hexaphosphate (Sigma Aldrich), glycerol (OmniPur) as the electron donor, and uranyl acetate (Spectrum) as follows: 10 mM phytate only; 10 mM phytate and 10 mM glycerol; 10 mM phytate and 200 μM U(VI); or 10 mM phytate, 10 mM glycerol, and 200 μM U(VI). Glycerol was chosen as electron donor as it has been used successfully to stimulate the formation of uranium phosphate minerals by the ORFRC microbial communities (Beazley et al., 2011; Salome et al., 2013). In addition, pH 7.0 incubations were initially equilibrated with 10 mM NaHCO_3 to reflect the composition of natural waters are circumneutral pH and inhibit schoepite precipitation. Although the phytate concentration used in the incubations was approximately two orders of magnitude higher than in

typical soils (Turner, 2007), the concentration of uranium used was also high compared to the field conditions in the pH range investigated (Gu et al., 2005). Reactors were maintained aerobically in the dark by agitating unsealed flasks at 200 rpm and 30 °C using a Lab-Line 3525 shaker incubator (Martinez et al., 2007). At each time point, part of a 1.5 mL filtered aliquot (0.2 µm, PES Puradisc, Whatman) was preserved in 2% trace metal grade nitric acid for uranium analysis, and the remainder was frozen (-20 °C) until analysis of inorganic phosphate (ΣPO_4^{3-}) and inositol phosphates. Following incubation, sediments were collected and divided for characterization by solid-phase sequential extraction, synchrotron X-ray diffraction spectroscopy (XRD), and X-ray absorption spectroscopy (XAS). Samples for XAS were preserved at -80°C in 50 mL centrifuge tubes until transport to the Stanford Synchrotron Radiation Lightsource (SSRL). Just before analysis, the sediments were thawed, loaded into windowed Lexan sample holders, and sealed with Kapton tape.

2.3 Analytical Techniques

Dissolved uranium was measured with an Agilent 7500a Series inductively-coupled plasma mass spectrometer (ICP-MS) (Salome et al., 2013). To prevent the interference of organophosphate compounds on conventional spectrophotometric techniques (Salome et al., 2013), total dissolved phosphate (ΣPO_4^{3-}) was quantified by ion chromatography using a Dionex GP-50 HPLC pump and conductivity detector (Dionex, CD-20) coupled to an Analytical Instrument Systems, Inc. integrator (LCC 100). An anion exchange analytical column (Dionex AS14, 4 x 250mm) and guard column (Dionex AG14, 4 x 50mm) were used in line with an AMMS-300 (4-mm, Dionex) suppressor. The operating conditions included a 1 mM NaHCO_3 and 3 mM Na_2CO_3 buffer in 10% acetonitrile eluent with a 1 mL min⁻¹ flow rate and a 25 mN H_2SO_4 regenerant. Errors reported for uranium and phosphate measurements represent the

standard deviation between triplicate incubations and the analytical error on duplicate measurements. Inositol hexaphosphate (IP_6), inositol pentaphosphate (IP_5), inositol tetrakisphosphate (IP_4), inositol triphosphate (IP_3), and inositol bisphosphate (IP_2) (standards from Sigma Aldrich) were quantified using a modified chromatographic separation coupled to suppressed spectrophotometric detection at 500 nm (Rounds and Nielsen, 1993) with a Dionex ICS-3000 dual pump chromatography system. Separation was achieved with an OmniPac PAX-100 guard column (4 x 40 mm), and eluent flow conditions included a 23 minute linear gradient (0.5 mL/min) from 0.01 M 1-methylpiperazine (pH 4.0) to 0.38 M NaNO_3 in 0.01 M 1-methylpiperazine (pH 4.0). Following separation, 0.015% (w/v) $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in 0.15% (w/v) sulfosalicylic acid (pH 1.8) was introduced (0.5 mL/min) as post-column reagent through a reaction coil to ensure thorough mixing. Complexation of the deep purple iron sulfosalicylate complex by the phosphate groups on the inositol ring suppresses its absorption at 500 nm in a proportion that is a function of the concentration of each inositol species. Reported errors for IP_x ($x = 1-6$) species include analytical error and standard variation between triplicate incubations.

The solid phase speciation of uranium was quantified at the end of the incubation using a modified sequential extraction technique (Tessier et al., 1979). The following procedure was performed sequentially: (1) 1.0 M MgCl_2 in 10 mM nitrilotriacetic acid (NTA) (pH 4.5) was added and agitated at 20°C for 1 hour to extract the loosely adsorbed fraction; (2) 1.0 M acetic acid (pH 5.0) was added and agitated at 20°C for 5 hours to dissolve uranium minerals; (3) 0.04 M $\text{NH}_2\text{OH} \cdot \text{HCl}$ in 25% (v/v) acetic acid was agitated at 96°C for 6 hours to remove Fe- and Mn-associated uranium; (4) 1.5 mL of 0.02 M HNO_3 and 2.5 mL of 30% H_2O_2 (pH 2.0) were agitated at 96°C for 2 hours, a second 1.5 mL aliquot of 30% H_2O_2 (pH 2.0) was added and agitated at 96°C for 3 hours, and a third 5 mL aliquot of 2.5 M NH_4OAc in 20% (v/v) HNO_3

was agitated at 20°C for 1 hour to extract the uranium fraction bound to organics; and (5) 15.8 M HNO₃ at 85°C was reacted for 3 hours to extract the residual fraction. After each extraction step, samples were centrifuged, and supernatants were filtered (0.2 µm, PES Puradisc Whatman) and reserved for uranium analysis. Control experiments indicate recovery efficiencies of greater than 80% for pure mineral phases (autunite) and uranium adsorbed onto lepidocrocite (not shown).

Sediment samples were characterized by synchrotron X-ray diffraction (XRD) and absorption (XAS) spectroscopy at the Stanford Synchrotron Radiation Lightsource (SSRL). Unfortunately, no usable data was collected by synchrotron XRD as the concentration of uranium in the sediments was too low to be detected. Uranium L_{III}-edge XAS spectra were collected at SSRL beam line 4-1 using a focused X-ray beam with a 23 keV harmonic rejection cutoff and a 13 element Ge detector. The incident energy was selected with a Si(220) monochromator crystal and data was collected using fluorescence detection. Extended X-ray absorption fine structure (EXAFS) data for each incubation treatment was fitted using SIXPACK (Webb, 2005), and phase and amplitude files for EXAFS fittings were created with FEFF7 (Ankudinov et al., 1998; Zabinsky et al., 1995). The axial oxygen coordination number (N) for all modeled treatments was set at two. Since the Debye-Waller factors (σ) correlated highly with coordination numbers (N), σ 's for some shells were each fixed at their average values, and the U=O_{ax}=U=O_{ax} transoxido multiple scattering path (Hudson et al., 1996) was included in all fits. Due to the similar backscattering intensities and phases of Mn and Fe in EXAFS, a “sum” of Fe/Mn like neighbors was reported for all treatments rather than distinct Fe and Mn shells (Salome et al., 2013).

3. RESULTS

3.1 Abiotic Precipitation of Uranium and Phytate

Equilibration of increasing concentrations of phytate with 200 μ M dissolved uranium revealed concentration-dependent uranyl solubilities at both pH 5.5 and 7.0 (Figure 1B), likely due to the highly negative charge of phytate at both pHs (Figure 1A). At aqueous $[\text{IP}_6]:[\text{U}]$ ratios below 15:1, uranium formed precipitates with IP_6 at both pH 5.5 and 7.0 (Figure 1B). At or above $[\text{IP}_6]:[\text{U}]$ ratios of 15:1, however, uranium predominantly remained in solution at both pHs (Figure 1B). These results indicate that any artifact from the precipitation of uranium phytate complexes in the incubations should be avoided in excess phytate. Thus, IP_6 concentrations of 10 mM were used in all the incubations conducted with 200 μ M U(VI) to ensure that the abiotic precipitation of uranium was minimized.

3.2 Phytate Hydrolysis and Speciation during Sediment Slurry Incubations

The pH of all reactors remained constant at the buffered pH for the duration of the incubations (data not shown). Interestingly, no inorganic phosphate was detected in any treatment at pH 7.0 (Figure 2), and IP_6 concentrations remained constant at ~ 9 mM for the duration of the experiments (Figure 3A-D). In contrast, all phytate-amended sediment slurry incubations at pH 5.5 liberated inorganic phosphate, although at different rates and to different extent depending on the treatment (Figure 2). In the absence of uranium, an 8 day difference in lag phase for the initial release of extracellular phosphate was observed in pH 5.5 reactors amended with both phytate and glycerol compared to identical reactors amended with phytate only (Figure 2). Once this initial limitation was overcome, however, inorganic phosphate was liberated at a comparable net rate in reactors amended with phytate and glycerol (20.5 ± 5.1 mM d^{-1}) and reactors amended with phytate but no external electron donor (13.5 ± 2.6 mM d^{-1}) (Table

1, Figure 2), suggesting similar organophosphate hydrolysis pathways in each of these conditions. Surprisingly, the difference in lag phase for the onset of inorganic phosphate production between pH 5.5 phytate-amended reactors with and without glycerol was eliminated upon introduction of 200 μM U(VI) (Figure 2). In fact, orthophosphate production in uranium-amended reactors containing phytate but no exogenous electron donor began 2 days before uranium-amended reactors containing both phytate and glycerol (Figure 2). In addition, net orthophosphate production rates in pH 5.5 reactors containing both phytate and uranium decreased by more than threefold compared to identical reactors without uranium, regardless of the presence ($5.6 \pm 0.4 \text{ mM d}^{-1}$) or absence ($4.6 \pm 0.3 \text{ mM d}^{-1}$) of external electron donor (Table 1, Figure 2).

As expected, the accumulation of aqueous orthophosphate at pH 5.5 was coupled to consumption of IP_6 in both treatments (Figure 3A-B), confirming the existence of phytate-metabolizing subsurface microorganisms at the ORFRC. However, in pH 5.5 reactors without uranium, a lag phase of a few days was observed between the onset of phytate hydrolysis and the accumulation of dissolved inorganic phosphate reactors amended with phytate (Figure 2 and 3A) compared to reactors amended with both phytate and glycerol (Figure 2 and 3B). Despite displaying similar orthophosphate production rates, the IP_6 consumption rate in pH 5.5 reactors with phytate and glycerol, neglecting lag phases ($2.5 \pm 0.58 \text{ mM d}^{-1}$) (Table 1, Figure 3B), was much higher than in identical reactors without exogenous electron donor ($0.92 \pm 0.22 \text{ mM d}^{-1}$) (Table 1, Figure 3A). In addition, intermediate inositol phosphates did not accumulate significantly in these conditions in the absence of uranium (Figure 3A-B) compared to similar incubations conducted in the presence of uranium, which displayed successive accumulation of IP_5 , IP_4 , and IP_3 in various concentrations depending on the presence of external electron donor

(Figure 3C-D). Finally, neglecting any lag phases, IP₆ consumption rates in pH 5.5 reactors amended with uranium and phytate apparently doubled ($1.7 \pm 0.38 \text{ mM d}^{-1}$) compared to identical reactors without uranium ($0.92 \pm 0.22 \text{ mM d}^{-1}$) (Table 1, Figure 3A and C), while IP₆ consumption rates in pH 5.5 reactors amended with uranium, phytate, and glycerol ($2.9 \pm 0.21 \text{ mM d}^{-1}$) were comparable to identical reactors without uranium ($2.5 \pm 0.58 \text{ mM d}^{-1}$) (Table 1, Figure 3B and D). The differences between IP₆ consumption rates without glycerol should be considered carefully, however, as the temporal resolution of the IP₆ measurements in the treatment without uranium was not high enough in the period of consumption to determine rates with high accuracy.

3.3 Transformation of Uranium during Sediment Slurry Incubations

Importantly, uranium was not released from Area 3 sediments in incubations amended with phytate but not uranium (data not shown), suggesting both a lack of ligand-mediated dissolution of uranium minerals and desorption of sorbed uranium species. Although $200 \text{ } \mu\text{M U(VI)}$ was introduced into each uranium-amended reactor, significantly less dissolved uranium was measured within minutes after inoculation. Only $60 \text{ } \mu\text{M}$ aqueous uranium was detected after 30 minutes in the pH 5.5 incubations without phytate, whereas $110 \text{ } \mu\text{M}$ was detected in pH 5.5 reactors amended with phytate (Figure 4A). Uranium concentrations in controlled incubations (i.e., no glycerol or phytate) at pH 5.5 decreased only slightly from their initial values over the first 8 days (Figure 4A), suggesting that uranium sorption was initially fast but incomplete. In contrast, 97% of the initial aqueous uranium was removed from solution by day 8 in pH 5.5 incubations containing phytate, regardless of the presence of electron donor (Figure 4A). Concurrently, relatively low concentrations of inorganic phosphate were produced in solution during the first week of the incubations with phytate, irrespective of the presence of electron

donor (Figure 4B). In pH 7.0 reactors amended with 200 μM U(VI), aqueous uranium concentrations averaged $\sim 130 \mu\text{M}$ initially and were not significantly affected by the presence or absence of phytate (Figure 4A). After the initial sampling point, however, aqueous uranium in pH 7.0 reactors without phytate rapidly decreased to $2 \mu\text{M}$, whereas dissolved uranium in pH 7.0 reactors amended with both uranium and phytate gradually decreased from $130 \mu\text{M}$ to $70 \mu\text{M}$ over the duration of the incubations (Figure 4A). The behavior of dissolved uranium in the incubations amended with glycerol, phytate, and uranium mirrored that of the incubations conducted without exogenous electron donor, albeit in slightly higher concentrations (Figure 4A). The drastic drop in aqueous uranium concentration in pH 7.0 reactors without phytate was followed at day 5 by a brief remobilization and gradual removal down to $2 \mu\text{M}$ by the end of the incubations (Figure 4A).

3.4 Solid-phase Uranium Characterization

Although sequential extractions of ORFRC sediments incubated at pH 5.5 and 7.0 in the presence of elevated uranium concentrations generally provided high uranium recoveries, they were slightly better at pH 5.5 than pH 7.0 (Figure 5). These extractions revealed a significant shift in solid-phase uranium speciation between slurries with and without phytate. Without phytate, uranium was primarily associated with Mg^{2+} exchangeable, acetate extractable, and hydroxylamine extractable fractions at both pH 5.5 (Figure 5A) and 7.0 (Figure 5B). In turn, solid-phase uranium speciation shifted towards the more recalcitrant hydroxylamine extractable and peroxide extractable fractions upon introduction of phytate at both pHs (Figure 5). Solid-phase extractions also revealed that 50% of uranium was strongly adsorbed (i.e., hydroxylamine extractable) at pH 7.0 in the presence of phytate (Figure 5B) compared to only 30% at pH 5.5 (Figure 5A). Simultaneously, 35% of uranium was found in the peroxide extractable fraction of

the pH 7.0 incubations with little or no phytate hydrolysis (Figure 5B), whereas 60% of uranium was extracted by peroxide in pH 5.5 incubations.

Examination of solid-phase uranium by XANES revealed a uranium L_{III}-edge at ~17163 eV and a characteristic U(VI) shoulder between 17187 and 17200 eV in all treatments (Figure 6A), confirming that uranium existed as U(VI) in these aerobic incubations. Additionally, the k³-weighted EXAFS fittings confirmed the presence of an axial oxygen shell at 1.80 Å in all incubations (Figure 6B-C, Table 2). Split equatorial oxygen shells at 2.25 Å and 2.41 Å were required to improve EXAFS fittings in uranium-amended reactors without phytate at both pH (Table 2). However, only a single equatorial oxygen shell was required to improve the fit of uranium-amended reactors in the presence of phytate at both pH, regardless of the presence of external electron donor (Table 2). In turn, the inclusion of Mn or Fe EXAFS shells was required for all treatments (Figure 5, Table 2), suggesting a close interaction between uranium and manganese and iron oxides. Although phosphorus shells improved EXAFS fittings of pH 5.5 uranium-amended reactors with phytate, in which significant inorganic phosphate was produced (Figure 6B and C), a P-shell was not required to improve EXAFS fittings of solids from the pH 7.0 phytate-amended incubations (Table 2). Finally, a C-shell was required to improve the fittings of the incubations conducted at pH 7.0, reflective of the addition of dissolved inorganic carbon (DIC) to buffer the pH.

4.0 DISCUSSION

4.1 Solubility of Uranium in the Presence of Phytate

In natural waters (pH 2-10), phytate carries a highly negative charge of between -6 and -9 (Figure 1A) and, thus, is expected to interact strongly with positively charged uranyl hydroxide

complexes at or below circumneutral pH, providing carbonates are not present in significant concentrations (Turner et al., 2002). Accordingly, the apparent concentration-dependent solubility of uranyl in the presence of phytate at both pH 5.5 and 7.0 (Figure 1B) was likely caused by the interaction of the positively charged uranyl ion with negatively charged IP₆ (Figure 1A). Although uranium precipitated with IP₆ at aqueous [IP₆]:[U] ratios below 15:1, uranium primarily remained in solution when phytate concentrations increased to or above 3 mM ([IP₆]:[U] ≥ 15:1). Previous studies have demonstrated similar concentration-dependent uranyl solubility in the presence of other organophosphates (Beazley et al., 2007), and the increase in uranium solubility with elevated phytate concentration is thought to be caused by enhanced electronic repulsion as the negatively charged phytate concentration increases around the uranium phytate complex. These findings have important implications for the promotion of U(VI)-phosphate biomineralization by phytate, as the [IP₆]:[U] ratio likely determines whether a chemical (i.e., adsorption of U(VI) at low concentrations of phytate) or biological (i.e., biomineralization of U(VI) phosphate minerals at high concentrations of phytate) sequestration process dominates.

4.2 Phytate Degradation during the Sediment Incubations

Inorganic phosphate was produced in all pH 5.5 incubations amended with phytate (Figure 2). However, the rate and extent of the net release of phosphate in solution varied according to the slurry treatment (Figure 2). If phytate degradation resulted from chemical hydrolysis, no differences in phosphate production should be observed in incubations amended with glycerol and/or uranium compared to their unamended controls. These characteristic differences in orthophosphate production, therefore, suggest that phytate degradation likely resulted from biologically-mediated phytate hydrolysis, possibly induced by phosphate starvation

conditions (Mukhametzyanova et al., 2012). Although these findings have to be confirmed, the phytases involved are likely acid phytases, which are typically activated between pH 2.0 and 6.0 (Rao et al., 2009). In contrast, pH 7.0 reactors did not produce inorganic phosphate (Figure 2), and 90% of the IP₆ added to the reactors remained in solution over the duration of the incubations (Figure 3A-D). Phytate adsorbs strongly to iron oxides at this pH (Degroot and Golterman, 1993; Johnson et al., 2012), suggesting that 10% phytate (~ 1 mM) was removed by adsorption onto ORFRC sediments. At such high concentration, phytate should also outcompete inorganic phosphate for sediment sorption sites (Degroot and Golterman, 1993). Thus, the lack of inorganic phosphate in solution suggest either that the small fraction of inorganic phosphate produced during phytate hydrolysis may have been consumed by incorporation into biomass or that phytate hydrolysis at pH 7.0 did not occur due to the absence of organisms carrying alkaline phytases or to a calcium limitation, as calcium is required for alkaline phytase activity (Ha et al., 2000). In any case, the lack of significant phytate hydrolysis at pH 7.0 suggests that phytate hydrolysis by the ORFRC natural microbial community is highly sensitive to subsurface geochemical conditions (i.e., pH, Ca²⁺), which is not surprising as phytase enzymes active at acidic, circumneutral, and/or alkaline pH are expressed by different genes and display different catalytic mechanisms (Oh et al., 2004).

An eight day difference in lag phase for the initial release of extracellular phosphate was observed in pH 5.5 reactors amended with phytate and glycerol but no uranium compared to identical reactors amended with phytate only (Figure 2). As only endogenous electron donors were available in incubations that did not receive uranium or glycerol amendments, this lag phase suggests that the activity of the natural microbial community was limited by electron donor availability resulting in delayed phytate mineralization. The onset of phytate hydrolysis

and orthophosphate accumulation was also delayed in reactors without uranium or glycerol (Figure 2 and 3A) relative to identical treatments with glycerol (Figure 2 and 3B), suggesting that sorption of phosphate to ORFRC sediments in the glycerol-amended incubations was limited by competitive sorption of glycerol (Liu et al., 2009; Violante and Pigna, 2002). In addition, the accumulation of inositol intermediates (IP₂-IP₅) was not evident in any of these reactors, regardless of the presence of external electron donor (Figure 3A-B), and mass balance calculations indicate that the sole inositol phosphate species not analyzed (IP₁) did not constitute a significant fraction of inositol phosphate intermediates in these incubations (1-12% of total P). Although IP₆ hydrolysis requires catalysis by phytase enzymes above pH 1 (Irving and Cosgrove, 1974), hydrolysis of lower inositol phosphates may be catalyzed by phytase enzymes and/or non-specific phosphomonoesterase (phosphatase) enzymes (Turner et al., 2002). These considerations suggest that IP₆ to IP₅ conversion by phytase enzymes could also have initiated glycerol-independent non-specific phosphatase enzyme production that facilitated dephosphorylation of intermediate inositol phosphate species.

Previous studies have proposed that microbial phosphatases are overexpressed in contaminated environments as detoxification mechanism resulting from exposure to heavy metals (Macaskie et al., 1994; Montgomery et al., 1995; Powers et al., 2002; Sobecky et al., 1996; Yong and Macaskie, 1995). The disparity in the lag phase of inorganic phosphate production between pH 5.5 phytate-amended reactors with and without glycerol was not only eliminated, but unexpectedly reversed in the presence of 200 μ M U(VI) (Figure 2). In addition, IP₆ consumption rates apparently doubled (1.7 ± 0.38 mM d⁻¹) in pH 5.5 uranium-amended reactors with phytate but no glycerol compared to identical reactors without uranium (0.92 ± 0.22 mM d⁻¹) (Table 1, Figure 3A and C), while these rates were comparable in uranium-

amended reactors with phytate and glycerol ($2.9 \pm 0.21 \text{ mM d}^{-1}$) compared to identical reactors without uranium ($2.5 \pm 0.58 \text{ mM d}^{-1}$) (Table 1, Figure 3B and D). As divalent cations advertently affect the activity of acid phytases (Oh et al., 2004), uranyl was likely not directly responsible for the increase in phytate hydrolysis. The increase in IP_6 consumption rate combined with the disappearance of the lag phase for the onset of orthophosphate production upon uranium addition, therefore, suggest that microbial communities may have responded to uranium stress by expressing phytase enzymes. Indeed, previous studies have demonstrated upregulation of phytase genes by *Caulobacter crescentus* exposed to uranium stress (Hu et al., 2005; Yung et al., 2014). The apparent increase in phytate hydrolysis by ORFRC microorganisms in the presence of glycerol but no uranium, however, indicates that electron donor limitation may also affect bacterial phytate metabolism.

The threefold decrease in net orthophosphate production rates in pH 5.5 reactors containing both phytate and uranium compared to identical reactors without uranium, regardless of the presence of external electron donor (Figure 2), was probably due to an alteration of the kinetics of hydrolysis of IP_5 through IP_2 in the presence of uranium as evidenced by the significant accumulation of intermediate inositol phosphates in these conditions (Figure 3C-D) compared to identical incubations without uranium (Figure 3A-B). The decreased transformation rates of the intermediate inositol phosphates in the presence of uranium at pH 5.5 suggest an apparent uranium toxicity effect on the ORFRC microbial community expressed either through the inhibition of phosphatase production and/or activity or through a shift in the dominant enzyme catalyzing IP_x ($x = 1-5$) hydrolysis from phosphatase to phytase. Given the complexity of the production and consumption kinetics of intermediate inositol phosphates, it is

difficult to distinguish between these two possible explanations for the observed decrease in IP_x ($x = 2-5$) transformation rates.

4.3 Fate of Uranium during the Sediment Incubations

In all uranium amended reactors, measured uranium concentrations decreased significantly from the initially added 200 μ M within minutes after inoculation (Figure 4A). In contrast to the the pH 7.0 incubations which displayed similar dissolved uranium concentrations initially, the presence of excess phytate in the pH 5.5 incubations resulted in a much higher initial dissolved uranium concentration than in controlled incubations without phytate. These results can be explained partly because uranium solubility is enhanced by the formation of uranium phytate complexes at both pHs (Figure 1B) and partly because the abundance of negatively charged surface groups on the iron oxides at circumneutral pH prevents significant adsorption of the negatively charged uranyl phytate or carbonate complexes at pH 7.0 (Katsoyiannis, 2007). In addition, as phytate is highly negatively charged at $pH > 2$ (Figure 1A) and adsorbs strongly and irreversibly to iron oxides (Degroot and Golterman, 1993; Johnson et al., 2012) and other natural sorbents (e.g., aluminosilicates), the apparent sorption of ~ 1 mM phytate before uranium addition (Figure 3A-D) may have provided an additional mechanism to prevent adsorption of uranium onto the solid phase.

More importantly, the significant decrease in dissolved uranium ($\sim 97\%$) during the first week of the pH 5.5 incubations amended with phytate regardless of the presence of external electron donor (Figure 4A) along with the relatively low concentrations of orthophosphate accumulated in solution (Figure 4B) suggest that inorganic phosphate likely contributed to uranium removal from solution through formation of stable U(VI)-phosphate minerals (Beazley

et al., 2007; Beazley et al., 2011; Paterson-Beedle et al., 2010; Shelobolina et al., 2009), though the precipitation of U(VI)-IP_x (x = 1-6) minerals (Knox et al., 2008; Nash et al., 1998; Seaman et al., 2003), which may be further transformed to U(VI)-phosphate minerals (Nash et al., 1998), may have also contributed to uranium removal in these incubations. Thermodynamic equilibrium calculations (Table 3) indeed predict that 100% of uranium should be precipitated as autunite at pH 5.5 given the degree of orthophosphate production and absence of carbonates in this system. In contrast, the gradual and incomplete decrease in dissolved uranium concentration in the phytate-amended incubations at pH 7.0 regardless of the presence of external electron donor (Figure 4A), combined with the lack of phytate hydrolysis in these conditions (Figure 2 and 3A-D), indicate the formation of aqueous U(VI)-phytate complexes increases uranium solubility by inhibiting uranium adsorption to ORFRC sediments. The lack of carbonates in the pH 5.5 incubations does not prevent comparison of these two sets of incubations, as carbonates should have little impact of U(VI) speciation at pH 5.5 given their low concentrations and weaker complexing properties with metals compared to phytate (Crea et al., 2008; Cigala et al., 2011). Considering the complete removal of uranium from solution during the first 24 hours in the absence of phytate but presence of carbonates at pH 7.0 (Figure 4A), these findings suggest that the presence of phytate also prevents sequestration of uranium via schoepite precipitation. Overall, the enhanced removal of dissolved uranium in phytate-amended reactors at pH 5.5, which display significant inorganic phosphate production from IP₆ hydrolysis compared to identical reactors at pH 7.0, demonstrates the efficiency of U(VI)-phosphate biomineralization promoted by phytate hydrolysis in low pH ORFRC sediments.

The solid phase extraction and XAS data obtained from the sediment at the end of the incubations can be combined with the dissolved uranium concentrations to identify the fate of

uranium in the solid phase. The apparent shift in solid-phase uranium speciation from the magnesium and acetate fractions in the initial sediment to the hydroxylamine and peroxide fractions in the sediment incubated in the presence or absence of phytate at both pH 5.5 and 7.0 (Figure 5) indicates that phytate addition triggers a change in the dominant uranium removal mechanism, regardless of the presence or absence of carbonates, even when inorganic phosphate is not produced. Previous studies investigating U(VI)-phosphate biomineralization also demonstrated significant hydroxylamine-extractable uranium in Oak Ridge sediments containing U(VI)-phosphate precipitates attributable either to the presence of strongly sorbed U(VI) or U(VI)-phosphate precipitates incorporated into iron oxides (Beazley et al., 2011; Salome et al., 2013). In the present incubations, the initial adsorption of both phytate (Figure 3) and uranium (Figure 4) indicates that ternary $=\text{Fe}-\text{IP}_6-\text{UO}_2^{2+}$ surface complexes may have contributed significantly to the hydroxylamine-extractable uranium fraction quantified at the end of the incubations. In addition, chemical control experiments (data not shown) indicate that U(VI)- IP_x ($x = 2-6$) precipitates constitute the majority of the observed peroxide-extractable uranium fraction in these experiments.

Comparison of dissolved uranium data in the phytate-amended incubations (Figure 4) reveals that 40% more uranium was removed in pH 5.5 incubations that released 40 mM inorganic phosphate than in the pH 7.0 incubations that did not produce orthophosphate. The additional uranium removed at pH 5.5 can be attributed to a mechanism other than adsorption and/or diffusion of uranium into the crystal lattice of mineral phases. The fraction of strongly adsorbed (i.e. hydroxylamine extractable) uranium was higher while the fraction of uranium associated with the peroxide extractable fraction was lower in pH 7.0 incubations amended with phytate compared to otherwise identical incubations at pH 5.5 (Figure 5). If the removal of

uranium was mainly due to the formation of U-IP₆ precipitates, the peroxide extractable fraction of uranium should be similar at pH 5.5 and 7.0 given the highly negative charge on IP₆ at both pHs (Figure 1A). These observations, therefore, suggest that approximately 35% of uranium was chemically precipitated with IP₆ at pH 7.0 (Figure 5B), while 70% of uranium was precipitated as a mixture of U-IP_x (x = 2-6) and U(VI)-PO₄³⁻ minerals at pH 5.5 (Figure 5A).

The presence of solid-phase associated U(VI) was confirmed by examination of solid-phase uranium by XANES. Concurrently, the split equatorial oxygen shells at 2.25 Å and 2.41 Å required to improve EXAFS fittings in uranium-amended reactors without phytate at both pH (Table 2) may indicate the presence of schoepite (Allen et al., 1996) or U(VI) ternary surface complexes on iron or manganese oxides with carbonates or other complexing ligands (Waite et al., 1994; Webb et al., 2006) in agreement with the rapid (< 24 hours) removal of dissolved uranium observed at both pHs (Figure 4A). Rather than the split equatorial shells observed in reactors without phytate, only a single equatorial oxygen shell was required to improve the fit of uranium-amended reactors in the presence of phytate at both pHs (Table 2). These findings indicate the presence of phytate may drive a shift in the dominant uranium removal mechanism between these treatments from surface-mediated ternary complex formation to solution-mediated precipitation reactions, even when no phytate hydrolysis occurs. This shift in removal mechanism is also reflected by the shift in solid-phase speciation between pH 7.0 reactors with and without phytate determined by sequential extractions (Figure 5B). Despite the presence of significant Al(III) hydroxides in Area 3 sediments (Wu et al., 2006a), past studies have demonstrated particulate uranium to be associated primarily with iron oxides, mixed manganese and iron oxides, and phosphate precipitates (Stubbs et al., 2006; Wu et al., 2006a). The required inclusion of Mn or Fe but not Al EXAFS shells in all treatments seems to confirm that sorption

to manganese or iron oxides represents a prevalent uranium sink in this system (Figure 5, Table 3), even in phytate-amended reactors where precipitation of U-phosphate phases is important. The required inclusion of phosphorus shells to improve the EXAFS fittings of pH 5.5 uranium-amended reactors with phytate (Table 2) suggests that solid-phase uranium in pH 5.5 reactors was likely associated with phosphate through a combination of ternary $=\text{Fe}-\text{IP}_6-\text{UO}_2^{2+}$ surface complexes, U(VI)-phosphate precipitates, and U(VI)-phytate precipitates. For pH 7.0 reactors, however, addition of a phosphorus shell did not improve the fit (Table 2), indicating that U(VI)- IP_6 precipitates may not represent the dominant form of solid U at this pH. Likely, both uranium sorption to ORFRC sediments and precipitation of schoepite contributed to uranium removal in the pH 7.0 incubations.

4.4. Environmental Implications

These findings have important environmental implications. First, the biomineralization of U(VI) phosphates has been proposed to represent a detoxification mechanism for microorganisms present in uranium-contaminated environments. As these environments are often associated with acidic pHs as a result of the presence of nitric acid used to extract uranium from ore, it appears logical that acid phytases but not alkaline phytases are activated by the presence of uranium. Second, as the hydrolysis of phytate by phytases generates intermediate inositol phosphate species that can be degraded by natural phosphatases (Turner et al., 2002), it is possible that a diverse microbial community may be stimulated by the hydrolysis of phytate. Although the present study reveals that uranium alters the overall hydrolysis of phytate

503 compared to similar incubations without uranium, the generation of vast concentrations of
504 inorganic phosphate in solution may be able to locally remediate the uranium problem and
505 simultaneously help the microbial community recover after removal of uranium via precipitation
506 of U(VI)-phosphate minerals. The production of large excess inorganic phosphate compared to
507 uranium concentrations in these incubations also indicates that phytate hydrolysis is efficient and
508 suggests that the natural phytate concentration of soils may be sufficient to promote the
509 biomineralization of uranium.

510 Simultaneously, the results of this study may have important ramifications for the
511 bioremediation of uranium-contaminated environments. Although bioreduction has been
512 extensively studied as a uranium bioremediation technique, the instability of the uraninite
513 mineral product, particularly in fluctuating redox environments, warrants investigation of
514 alternative uranium immobilization strategies. The biomineralization of U(VI)-phosphate
515 minerals may represent an appealing remediation technique for *in situ* uranium immobilization as
516 it may occur in both aerobic (Beazley et al., 2007; Macaskie et al., 1995; Martinez et al., 2007;
517 Montgomery et al., 1995; Shelobolina et al., 2009) and anaerobic conditions (Beazley et al.,
518 2009; Shelobolina et al., 2009) as well as in elevated nitrate and low pH conditions (Beazley et
519 al., 2011; Salome et al., 2013) where bioreduction is not favorable (Belli and Taillefert, 2016).
520 Thus far, U(VI)-phosphate biomineralization has primarily been demonstrated using synthetic
521 organophosphate sources such as glycerol-2-phosphate. Utilizing phytate in uranium
522 bioremediation strategies may present several advantages over previously studied compounds.
523 First, phytate, a naturally occurring organophosphate in surface soils, may potentially yields six
524 moles of orthophosphate per mole of phytate and promote the natural attenuation of uranium
525 contaminations via the biomineralization of uranium phosphate minerals. Second, phytate can be

526 purified inexpensively from wheat products (Abebe et al., 2007) if exogenous sources are
527 required for bioremediation. Third, as the microbial community hydrolyzed the majority of the
528 high phytate concentrations used in the incubations, phytate amendments could be optimized in
529 bioremediation strategies to maximize the coverage of the contaminated area while
530 simultaneously keeping production of the potential contaminant by-product orthophosphate to a
531 minimum. Fourth, the lower net rates of transformation of inositol phosphate intermediates and
532 the slower kinetics of production of inorganic phosphate in the presence of elevated uranium
533 could allow time for phytate and its inositol phosphate intermediates to migrate within
534 contaminant plumes before hydrolysis occurs and, thus, increase the remediated surface area.
535 Fifth, while phytate sorption may limit its transport, sorbed IP_6 may also represent a long term
536 source of inorganic phosphate to contaminated environments. Finally, phytate may chemically
537 precipitate with uranium (Knox et al., 2008; Nash et al., 1998; Seaman et al., 2003) depending on
538 its concentration (Figure 1), further decreasing aqueous uranium concentrations. As phytate
539 hydrolysis results in the precipitation of uranium, previous concerns over increased uranium
540 mobility in contaminated sediments upon phytate amendment (Seaman et al., 2003) seem to be
541 unfounded. In turn, two important aspects have to be considered before phytate amendments can
542 be made in contaminated environments. First, uranium solubility may be enhanced at
543 circumneutral pH in the presence of elevated concentrations of phytate if phytate hydrolysis does
544 not occur, regardless of the concentration of DIC (Figure 1). Although phytase enzymes are
545 commonly found in soils and aquatic systems (Jorquera et al., 2008; Lim et al., 2007),
546 preliminary studies must be conducted to ensure the indigenous microbial community of a
547 particular contaminated subsurface expresses phytase enzymes in geochemical conditions
548 representative of that site. Second, the U(VI)-phosphate by-product may be destabilized by

carbonates at circumneutral pH if their concentrations are high enough. Although U(VI)-phosphate minerals are found in a wide variety of soils (Sato et al. 1997), including those in the DOE sites in Oak Ridge, TN (Roh et al. 2000) and Hanford, WA (Arai et al. 2007) and the former Fernald Environmental Management Project site in Fernald, OH (Morris et al. 1996), such strategy may therefore be better suited for low pH environments where the influence of carbonates is minimized.

5.0 CONCLUSIONS

The large volume of uranium-contaminated groundwater and soils at DOE facilities across the United States warrants the development of creative in situ remediation techniques that are able to immobilize uranium in the solid phase over large areas. In this study, the biomineralization of U(VI)-phosphate minerals promoted by the addition of phytate, a natural organophosphate compound found in soils, was demonstrated in uranium-contaminated sediments of the ORFRC. To our knowledge, this is the first study investigating U(VI)-phosphate biomineralization promoted by phytate hydrolysis. The hydrolysis of phytate with the concomitant release of inorganic phosphate by indigenous subsurface microorganisms of ORFRC sediments occurred at low pH and was enhanced in the presence of uranium, likely as a response to uranium toxicity. At the same time, uranium stress decreased hydrolysis of intermediate inositol phosphate species and promoted the immobilization of uranium via ternary $=\text{Fe}-\text{IP}_6-\text{UO}_2^{2+}$ surface complex formation, precipitation of U(VI)-phytate minerals, and biomineralization of U(VI)-phosphate minerals. Interestingly, phytate hydrolysis was not observed in pH 7.0 incubations, suggesting that alkaline phytase was not produced by native ORFRC microorganisms or that calcium concentrations were not sufficient to support the activity of alkaline phytase. These findings have important environmental implications. First,

the abundance of plant-derived phytate in soils may promote the natural attenuation of uranium contaminations via the biomineralization of uranium phosphate minerals. Second, if the biomineralization of U(VI) phosphates acts as a detoxification mechanism for microorganisms, the large phosphate concentrations generated by phytate hydrolysis may be able to help the natural microbial community recover from any effects of the presence of uranium after U(VI)-phosphate precipitation. Third, promoting the biomineralization of U(VI)-phosphate minerals using phytate as organophosphate source may represent an inexpensive bioremediation alternative as phytate is easily extracted from wheat products and generates much higher concentrations of inorganic phosphates compared to other organophosphate compounds investigated for such applications. Fourth, although the hydrolysis of lower inositol derivatives by conventional phosphohydrolases may be affected by the presence of uranium, the slow hydrolysis of phytate and its inositol phosphate intermediates in the presence of uranium may facilitate its spreading over large surface areas and provide a long-term source of inorganic phosphorus. Finally, phytate may abiotically precipitate uranium and, therefore, does not always require phytases to be efficient. To determine the exact effect of uranium on the phytate-hydrolyzing microbial community and to elucidate the mechanism of phytate-hydrolysis by this community, however, pure cultures should be isolated. As ORFRC groundwater may be highly acidic with fluctuating redox conditions, the ability of the natural microbial community to hydrolyze phytate at lower pH or in nitrate-reducing conditions should also be examined.

ACKNOWLEDGEMENTS

This research was supported by the Office of Science (BER), US DOE Grant No. DE-FG02-04ER63906. Portions of this research were carried out at the Stanford Synchrotron

595 Radiation Lightsource, a national user facility operated by Stanford University on behalf of the
596 US DOE, Office of Basic Energy Sciences. We thank Dave Watson of Oak Ridge National
597 Laboratory for providing ORFRC sediment cores. We also thank the Associated Editor and four
598 anonymous reviewers for their constructive suggestions to improve this paper.

References

- 599 Abebe, Y., Bogale, A., Hambidge, K.M., Stoecker, B.J., Bailey, K. and Gibson, R.S. (2007)
600 Phytate, zinc, iron and calcium content of selected raw and prepared foods consumed in
601 rural Sidama, Southern Ethiopia, and implications for bioavailability. *J. Food Comp.*
602 *Anal.* 20, 161-168.
- 603 Alessi, D.S., Lezama-Pacheco, J.S., Stubbs, J.E., Janousch, M., Bargar, J.R., Persson, P. and
604 Bernier-Latmani, R. (2014) The product of microbial uranium reduction includes
605 multiple species with U(IV)-phosphate coordination. *Geochim. Cosmochim. Acta* 131,
606 115-127.
- 607 Allen, P.G., Shuh, D.K., Bucher, J.J., Edelstein, N.M., Palmer, C.E.A., Silva, R.J., Nguyen, S.N.,
608 Marquez, L.N. and Hudson, E.A. (1996) Determinations of uranium structures by
609 EXAFS: Schoepite and other U(VI) oxide precipitates. *Radiochim. Acta* 75, 47-53.
- 610 Ankudinov, A.L., Ravel, B., Rehr, J.J. and Conradson, S.D. (1998) Real-space multiple-
611 scattering calculation and interpretation of x-ray-absorption near-edge structure. *Phys.*
612 *Rev. B* 58, 7565.
- 613 Bai, J., Liu, C.X. and Ball, W.P. (2009) Study of Sorption-Retarded U(VI) Diffusion in Hanford
614 Silt/Clay Material. *Environ. Sci. Technol.* 43, 7706-7711.
- 615 Barnett, M.O., Jardine, P.M. and Brooks, S.C. (2002) U(VI) adsorption to heterogeneous
616 subsurface media: Application of a surface complexation model. *Environ. Sci. Technol.*
617 36, 937-942.
- 618 Beazley, M.J., Martinez, R.J., Sobecky, P.A., Webb, S.M. and Taillefert, M. (2007) Uranium
619 biomineralization as a result of bacterial phosphatase activity: Insights from bacterial
620 isolates from a contaminated subsurface. *Environ. Sci. Technol.* 41, 5701-5707.

621 Beazley, M.J., Martinez, R.J., Sobecky, P.A., Webb, S.M. and Taillefert, M. (2009)
622 Nonreductive Biomineralization of Uranium(VI) Phosphate Via Microbial Phosphatase
623 Activity in Anaerobic Conditions. *Geomicrobiol. J.* 26, 431-441.

624 Beazley, M.J., Martinez, R.J., Webb, S.M., Sobecky, P.A. and Taillefert, M. (2011) The effect of
625 pH and natural microbial phosphatase activity on the speciation of uranium in subsurface
626 soils. *Geochim. Cosmochim. Acta* 75, 5648-5663.

627 Belli, K., Taillefert, M., 2016. Biogeochemical processes regulating the mobility of uranium in
628 sediments, in: Rinklebe, J., Knox, A.S., Paller, M. (Eds.), *Trace elements in waterlogged*
629 *soils and sediments*. CRC Press, a Taylor & Francis Company, pp. 185-223.

630 Bernier-Latmani, R., Veeramani, H., Vecchia, E.D., Junier, P., Lezama-Pacheco, J.S., Suvorova,
631 E.I., Sharp, J.O., Wigginton, N.S. and Bargar, J.R. (2010) Non-uraninite Products of
632 Microbial U(VI) Reduction. *Environ. Sci. Technol.* 44, 9456-9462.

633 Boyanov, M.I., Fletcher, K.E., Kwon, M.J., Rui, X., O'Loughlin, E.J., Loeffler, F.E. and
634 Kemner, K.M. (2011) Solution and Microbial Controls on the Formation of Reduced
635 U(IV) Species. *Environ. Sci. Technol.* 45, 8336-8344.

636 Brooks, S.C. (2001) Waste Characteristics of the Former S-3 Ponds and Outline of Uranium
637 Chemistry Relevant to NABIR Field Research Center Studies. NABIR FRC.

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

641 Cigala, R.M., Crea, F., De Stefano, C., Lando, G., Milea, D., Sammartano, S., 2010.
642 Electrochemical study on the stability of phytate complexes with Cu^{2+} , Pb^{2+} , Zn^{2+} , and
643 Ni^{2+} : A comparison of different techniques. *J. Chem. Eng. Data* 55, 4757-4767.

644 Costello, A.J.R., Glonek, T. and Myers, T.C. (1976) P-31 Nuclear magnetic resonance - pH
 645 titrations of myoinositol hexaphosphate. *Carbohydr. Res.* 46, 159-171.

646 Crea, F., De Stefano, C., Milea, D., Sammartano, S., 2008. Formation and stability of phytate
 647 complexes in solution. *Coord. Chem. Rev.* 252, 1108-1120.

648 Degroot, C.J. and Golterman, H.L. (1993) On the presence of organic phosphate in some
 649 camargue sediments-evidence for the importance of phytate. *Hydrobiol.* 252, 117-126.

650 DOE (1997) Linking Legacies - Connecting the Cold War Nuclear Weapons Production
 651 Processes to Their Environmental Consequences Office of Environmental Management -
 652 The U.S. Department of Energy Washington, D.C.

653 Finch, R. and Murakami, T. (1999) Systematics and paragenesis of uranium minerals. *Rev.*
 654 *Mineral. Geochem.* 38, 91-179.

655 Finneran, K.T., Housewright, M.E. and Lovley, D.R. (2002) Multiple influences of nitrate on
 656 uranium solubility during bioremediation of uranium-contaminated subsurface sediments.
 657 *Environ. Microbiol.* 4, 510-516.

658 Fletcher, K.E., Boyanov, M.I., Thomas, S.H., Wu, Q.Z., Kemner, K.M. and Löffler, F.E. (2010)
 659 U(VI) Reduction to Mononuclear U(IV) by *Desulfitobacterium* Species. *Environ. Sci.*
 660 *Technol.* 44, 4705-4709.

661 Fredrickson, J.K., Zachara, J.M., Kennedy, D.W., Liu, C.X., Duff, M.C., Hunter, D.B. and
 662 Dohnalkova, A. (2002) Influence of Mn oxides on the reduction of uranium(VI) by the
 663 metal-reducing bacterium *Shewanella putrefaciens*. *Geochim. Cosmochim. Acta* 66,
 664 3247-3262.

665 Grabias, E., Gladysz-Plaska, A., Ksiazek, A. and Majdan, M. (2014) Efficient uranium
 666 immobilization on red clay with phosphates. *Environ. Chem. Lett.* 12, 297-301.

667 Gu, B.H., Wu, W.M., Ginder-Vogel, M.A., Yan, H., Fields, M.W., Zhou, J., Fendorf, S., Criddle,
 668 C.S. and Jardine, P.M. (2005) Bioreduction of uranium in a contaminated soil column.
 669 Environ. Sci. Technol. 39, 4841-4847.

670 Ha, N.C., Oh, B.C., Shin, S., Kim, H.J., Oh, T.K., Kim, Y.O., Choi, K.Y. and Oh, B.H. (2000)
 671 Crystal structures of a novel, thermostable phytase in partially and fully calcium-loaded
 672 states. Nature Struct. Biol. 7, 147-153.

673 Han, R.P., Zou, W.H., Wang, Y. and Zhu, L. (2007) Removal of uranium(VI) from aqueous
 674 solutions by manganese oxide coated zeolite: discussion of adsorption isotherms and pH
 675 effect. J. Environ. Radioact. 93, 127-143.

676 Hsi, C.K.D. and Langmuir, D. (1985) Adsorption of uranyl onto ferric oxyhydroxides -
 677 Application of the surface complexation site-binding model. Geochim. Cosmochim. Acta
 678 49, 1931-1941.

679 Hu, P., Brodie, E.L., Suzuki, Y., McAdams, H.H. and Andersen, G.L. (2005) Whole-genome
 680 transcriptional analysis of heavy metal stresses in *Caulobacter crescentus*. J. Bacteriol.
 681 187, 8437-8449.

682 Hua, B. and Deng, B. (2008) Reductive Immobilization of Uranium(VI) by Amorphous Iron
 683 Sulfide. Environ. Sci. Technol. 42, 8703-8708.

684 Hudson, E.A., Allen, P.G., Terminello, L.J., Denecke, M.A. and Reich, T. (1996) Polarized x-
 685 ray-absorption spectroscopy of the uranyl ion: Comparison of experiment and theory.
 686 Phys. Rev. B 54, 156.

687 Irving, G.C. and Cosgrove, D.J. (1974) Inositol phosphate phosphatases of microbiological
 688 origin: Some properties of the partially purified phosphatases of *Aspergillus ficuum*
 689 NRRL 3135. Austr. J. Biol. Sci. 27, 361-368.

690 Istok, J.D., Senko, J.M., Krumholz, L.R., Watson, D., Bogle, M.A., Peacock, A., Chang, Y.J. and
691 White, D.C. (2004) In situ bioreduction of technetium and uranium in a nitrate-
692 contaminated aquifer. *Environ. Sci. Technol.* 38, 468-475.

693 Johnson, B.B., Quill, E. and Angove, M.J. (2012) An investigation of the mode of sorption of
694 inositol hexaphosphate to goethite. *J. Coll. Interf. Sci.* 367, 436-442.

695 Jorquera, M., Martinez, O., Maruyama, F., Marschner, P. and de la Luz Mora, M. (2008) Current
696 and future biotechnological applications of bacterial phytases and phytase-producing
697 bacteria. *Microbes and environments / JSME* 23, 182-191.

698 Katsoyiannis, I.A. (2007) Carbonate effects and pH-dependence of uranium sorption onto
699 bacteriogenic iron oxides: Kinetic and equilibrium studies. *J. Hazard. Mat.* 139, 31-37.

700 Kelly, S.D., Kemner, K.M., Carley, J., Criddle, C., Jardine, P.M., Marsh, T.L., Phillips, D.,
701 Watson, D. and Wu, W.M. (2008) Speciation of uranium in sediments before and after in
702 situ biostimulation. *Environ. Sci. Technol.* 42, 1558-1564.

703 Knox, A.S., Brimon, R.L., Kaplan, D.I. and Paller, M.H. (2008) Interactions among phosphate
704 amendments, microbes and uranium mobility in contaminated sediments. *Sci. Total*
705 *Environ.* 395, 63-71.

706 Langmuir, D. (1978) Uranium solution-mineral equilibria at low-temperatures with applications
707 to sedimentary ore-deposits. *Geochim. Cosmochim. Acta* 42, 547-569.

708 Langmuir, D. (1997) *Aqueous Environmental Geochemistry*. Prentice Hall, Upper Saddle River,
709 New Jersey.

710 Li, D., Seaman, J.C., Chang, H.S., Jaffe, P.R., van Groos, P.K., Jiang, D.T., Chen, N., Lin, J.R.,
711 Arthur, Z., Pan, Y.M., Scheckel, K.G., Newville, M., Lanzirotti, A. and Kaplan, D.I.

712 (2014) Retention and chemical speciation of uranium in an oxidized wetland sediment
 713 from the Savannah River Site. *J. Environ. Radioact.* 131, 40-46.

714 Liger, E., Charlet, L. and Van Cappellen, P. (1999) Surface catalysis of uranium(VI) reduction
 715 by iron(II). *Geochim. Cosmochim. Acta* 63, 2939-2955.

716 Lim, B.L., Yeung, P., Cheng, C. and Hill, J.E. (2007) Distribution and diversity of phytate-
 717 mineralizing bacteria. *ISME* 1, 321-330.

718 Liu, S.T., Musuku, S.R., Adhikari, S. and Fernando, S. (2009) Adsorption of glycerol from
 719 biodiesel washwaters. *Environ. Technol.* 30, 505-510.

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

722 Luo, W., Wu, W.-M., Yan, T., Criddle, C., Jardine, P., Zhou, J. and Gu, B. (2007) Influence of
 723 bicarbonate, sulfate, and electron donors on biological reduction of uranium and
 724 microbial community composition. *App. Microbiol. Biotechnol.* 77, 713-721.

725 Macaskie, L.E., Bonthron, K.M. and Rouch, D.A. (1994) Phosphatase-mediated heavy metal
 726 accumulation by a *Citrobacter* sp. and related enterobacteria. *FEMS Microbiol. Lett.* 121,
 727 141-146.

728 Macaskie, L.E., Hewitt, C.J., Shearer, J.A. and Kent, C.A. (1995) Biomass production for the
 729 removal of heavy-metals from aqueous-solutions at low pH using growth-decoupled cells
 730 of a *citrobacter* sp. *Inter. Biodeter. Biodegrad.* 35, 73-92.

731 Martin, C.J. and Evans, W.J. (1987) Phytic acid-divalent cation interactions. V. Titrimetric,
 732 calorimetric, and binding-studies with Cobalt(II) and Nickel(II) and their comparison
 733 with other metal-ions *J. Inorg. Biochem.* 30, 101-119.

734 Martinez, R.J., Beazley, M.J., Taillefert, M., Arakaki, A.K., Skolnick, J. and Sobecky, P.A.
 735 (2007) Aerobic uranium (VI) bioprecipitation by metal-resistant bacteria isolated from
 736 radionuclide- and metal-contaminated subsurface soils. *Environ. Microbiol.* 9, 3122-
 737 3133.

738 Meleshyn, A., Azeroual, M., Reeck, T., Houben, G., Riebe, B. and Bunnenberg, C. (2009)
 739 Influence of (Calcium-)Uranyl-Carbonate Complexation on U(VI) Sorption on Ca- and
 740 Na-Bentonites. *Environ. Sci. Technol.* 43, 4896-4901.

741 Missana, T., García-Gutiérrez, M. and Maffiotte, C. (2003) Experimental and modeling study of
 742 the uranium (VI) sorption on goethite. *J Coll. Interf. Sci.* 260, 291-301.

743 Montgomery, D.M., Dean, A.C.R., Wiffen, P. and Macaskie, L.E. (1995) Phosphatase
 744 production and activity in *Citrobacter freundii* and a naturally-occurring, heavy-metal-
 745 accumulating *Citrobacter* sp. *Microbiol.* 141, 2433-2441.

746 Mukhametzhanova, A., Akhmetova, A. and Sharipova, M. (2012) Microorganisms as phytase
 747 producers. *Microbiol.* 81, 267-275.

748 Murphy, W.M. and Shock, E.L. (1999) Environmental aqueous geochemistry of actinides, in:
 749 Burns, P.C.a.F., R. (Ed.), *Uranium: Mineralogy, Geochemistry and the Environment*.
 750 Mineralogical Society of America, Washington, D.C., pp. 221-254.

751 Nash, K.L., Jensen, M.P. and Schmidt, M.A. (1998) Actinide immobilization in the subsurface
 752 environment by in-situ treatment with a hydrolytically unstable organophosphorus
 753 complexant: Uranyl uptake by calcium phytate. *J. All. Comp.* 271, 257-261.

754 North, N.N., Dollhopf, S.L., Petrie, L., Istok, J.D., Balkwill, D.L. and Kostka, J.E. (2004)
 755 Change in bacterial community structure during in situ Biostimulation of subsurface

756 sediment cocontaminated with uranium and nitrate. *App. Environ. Microbiol.* 70, 4911-
757 4920.

758 O'Loughlin, E.J., Kelly, S.D., Cook, R.E., Csencsits, R. and Kemner, K.M. (2003) Reduction of
759 Uranium(VI) by mixed iron(II/iron(III) hydroxide (green rust): Formation of UO₂
760 nanoparticles. *Environ. Sci. Technol.* 37, 721-727.

761 Oh, B.C., Choi, W.C., Park, S., Kim, Y.O. and Oh, T.K. (2004) Biochemical properties and
762 substrate specificities of alkaline and histidine acid phytases. *App. Microbiol. Biotechnol.*
763 63, 362-372.

764 Paterson-Beedle, M., Readman, J.E., Hriljac, J.A. and Macaskie, L.E. (2010) Biorecovery of
765 uranium from aqueous solutions at the expense of phytic acid. *Hydrometal.* 104, 524-528.

766 Plathe, K.L., Lee, S.W., Tebo, B.M., Bargar, J.R. and Bernier-Latmani, R. (2013) Impact of
767 Microbial Mn Oxidation on the Remobilization of Bio-reduced U(IV). *Environ. Sci.*
768 *Technol.* 47, 3606-3613.

769 Powers, L.G., Mills, H.J., Palumbo, A.V., Zhang, C.L., Delaney, K. and Sobecky, P.A. (2002)
770 Introduction of a plasmid-encoded *phoA* gene for constitutive overproduction of alkaline
771 phosphatase in three subsurface *Pseudomonas* isolates. *FEMS Microbiol. Ecol.* 41, 115-
772 123.

773 Rao, D., Rao, K.V., Reddy, T.P. and Reddy, V.D. (2009) Molecular characterization,
774 physicochemical properties, known and potential applications of phytases: An overview.
775 *Crit. Rev. Biotechnol.* 29, 182-198.

776 Rounds, M.A. and Nielsen, S.S. (1993) Anion-exchange high-performance liquid
777 chromatography with post-column detection for the analysis of phytic acid and other
778 inositol phosphates. *J. Chromatogr. A* 653, 148-152.

779 Salome, K.R., Green, S.J., Beazley, M.J., Webb, S.M., Kostka, J.E. and Taillefert, M. (2013) The
780 role of anaerobic respiration in the immobilization of uranium through biomineralization
781 of phosphate minerals. *Geochim. Cosmochim. Acta* 106, 344-363.

782 Sanford, R.A., Wu, Q., Sung, Y., Thomas, S.H., Amos, B.K., Prince, E.K. and Loffler, F.E.
783 (2007) Hexavalent uranium supports growth of *Anaeromyxobacter dehalogenans* and
784 *Geobacter* spp. with lower than predicted biomass yields. *Environ. Microbiol.* 9, 2885-
785 2893.

786 Schecher, W.D. and McAvoy, D.C. (2001) MINEQL+: A Chemical Equilibrium Modeling
787 System, Version 4.5 for Windows, User's Manual. Research Software, Hallowell, Maine.

788 Seaman, J.C., Hutchison, J.M., Jackson, B.P. and Vulava, V.M. (2003) In situ treatment of
789 metals in contaminated soils with phytate. *J. Environ. Qual.* 32, 153-161.

790 Senko, J.M., Istok, J.D., Suflita, J.M. and Krumholz, L.R. (2002) In-situ evidence for uranium
791 immobilization and remobilization. *Environ. Sci. Technol.* 36, 1491-1496.

792 Senko, J.M., Mohamed, Y., Dewers, T.A. and Krumholz, L.R. (2005a) Role for Fe(III) minerals
793 in nitrate-dependent microbial U(IV) oxidation. *Environ. Sci. Technol.* 39, 2529-2536.

794 Senko, J.M., Suflita, J.M. and Krumholz, L.R. (2005b) Geochemical controls on microbial
795 nitrate-dependent U(IV) oxidation. *Geomicrobiol. J.* 22, 371-378.

796 Sharp, J.O., Lezama-Pacheco, J.S., Schofield, E.J., Junier, P., Ulrich, K.-U., Chinni, S.,
797 Veeramani, H., Margot-Roquier, C., Webb, S.M., Tebo, B.M., Giammar, D.E., Bargar,
798 J.R. and Bernier-Latmani, R. (2011) Uranium speciation and stability after reductive
799 immobilization in aquifer sediments. *Geochim. Cosmochim. Acta* 75, 6497-6510.

800 Shelobolina, E.S., Konishi, H., Xu, H.F. and Roden, E.E. (2009) U(VI) Sequestration in
801 Hydroxyapatite Produced by Microbial Glycerol 3-Phosphate Metabolism. *App. Environ.*
802 *Microbiol.* 75, 5773-5778.

803 Sitte, J., Akob, D.M., Kaufmann, C., Finster, K., Banerjee, D., Burkhardt, E.M., Kostka, J.E.,
804 Scheinost, A.C., Buchel, G. and Kuesel, K. (2010) Microbial Links between Sulfate
805 Reduction and Metal Retention in Uranium- and Heavy Metal-Contaminated Soil. *App.*
806 *Environ. Microbiol.* 76, 3143-3152.

807 Sobecky, P.A., Schell, M.A., Moran, M.A. and Hodson, R.E. (1996) Impact of a genetically
808 engineered bacterium with enhanced alkaline phosphatase activity on marine
809 phytoplankton communities. *App. Environ. Microbiol.* 62, 6-12.

810 Stubbs, J.E., Elbert, D.C., Veblen, D.R. and Zhu, C. (2006) Electron microbeam investigation of
811 uranium-contaminated soils from Oak Ridge, TN, USA. *Environ. Sci. Technol.* 40, 2108-
812 2113.

813 Tebo, B.M. and Obraztsova, A.Y. (1998) Sulfate-reducing bacterium grows with Cr(VI), U(VI),
814 Mn(IV), and Fe(III) as electron acceptors. *FEMS Microbiology Letters* 162, 193-198.

815 Tessier, A., Campbell, P.G.C. and Bisson, M. (1979) Sequential extraction procedure for the
816 speciation of particulate trace-metals. *Anal. Chem.* 51, 844-851.

817 Turner, B.L. (2007) Inositol phosphates in soil: Amounts, forms and significance of the
818 phosphorylated inositol stereoisomers in: Turner, B.L., Richardson, A.E., Mullaney, E.J.
819 (Eds.), *Inositol Phosphates: Linking Agriculture and the Environment*. CABI,
820 Wallingford, UK, pp. 186-208.

821 Turner, B.L., Paphazy, M.J., Haygarth, P.M. and McKelvie, I.D. (2002) Inositol phosphates in
822 the environment. *Phil. Trans. Roy. Soc. Lon. B* 357, 449-469.

823 Van Nostrand, J.D., Wu, L., Wu, W.-M., Huang, Z., Gentry, T.J., Deng, Y., Carley, J., Carroll,
824 S., He, Z., Gu, B., Luo, J., Criddle, C.S., Watson, D.B., Jardine, P.M., Marsh, T.L.,
825 Tiedje, J.M., Hazen, T.C. and Zhou, J. (2011) Dynamics of Microbial Community
826 Composition and Function during In Situ Bioremediation of a Uranium-Contaminated
827 Aquifer. *App. Environ. Microbiol.* 77, 3860-3869.

828 Violante, A. and Pigna, M. (2002) Competitive sorption of arsenate and phosphate on different
829 clay minerals and soils. *Soil Sci. Soc. Am. J.* 66, 1788-1796.

830 Wade, R. and DiChristina, T.J. (2000) Isolation of U(VI) reduction-deficient mutants of
831 *Shewanella putrefaciens*. *FEMS Microbiol. Lett.* 184, 143-148.

832 Waite, T.D., Davis, J.A., Payne, T.E., Waychunas, G.A. and Xu, N. (1994) Uranium(VI)
833 adsorption to ferrihydrite- application of a surface complexation model. *Geochim.*
834 *Cosmochim. Acta* 58, 5465-5478.

835 Wan, J.M., Tokunaga, T.K., Brodie, E., Wang, Z.M., Zheng, Z.P., Herman, D., Hazen, T.C.,
836 Firestone, M.K. and Sutton, S.R. (2005) Reoxidation of bio-reduced uranium under
837 reducing conditions. *Environ. Sci. Technol.* 39, 6162-6169.

838 Webb, S.M. (2005) SIXpack: a graphical user interface for XAS analysis using IFEFFIT. *Phys.*
839 *Script.* T115, 1011-1014.

840 Webb, S.M., Fuller, C.C., Tebo, B.M. and Bargar, J.R. (2006) Determination of uranyl
841 incorporation into biogenic manganese oxides using X-ray absorption spectroscopy and
842 scattering. *Environ. Sci. Technol.* 40, 771-777.

843 Wellman, D.M., Icenhower, J.P. and Owen, A.T. (2006) Comparative analysis of soluble
844 phosphate amendments for the remediation of heavy metal contaminants: Effect on
845 sediment hydraulic conductivity. *Environ. Chem.* 3, 219-224.

846 Wersin, P., Hochella, M.F., Persson, P., Redden, G., Leckie, J.O. and Harris, D.W. (1994)
847 Interaction between aqueous uranium(VI) and sulfide minerals - spectroscopic evidence
848 for sorption and reduction. *Geochim. Cosmochim. Acta* 58, 2829-2843.

849 Wu, W.M., Carley, J., Fienen, M., Mehlhorn, T., Lowe, K., Nyman, J., Luo, J., Gentile, M.E.,
850 Rajan, R., Wagner, D., Hickey, R.F., Gu, B.H., Watson, D., Cirpka, O.A., Kitanidis, P.K.,
851 Jardine, P.M. and Criddle, C.S. (2006a) Pilot-scale in situ bioremediation of uranium in a
852 highly contaminated aquifer. 1. Conditioning of a treatment zone. *Environ. Sci. Technol.*
853 40, 3978-3985.

854 Wu, W.M., Carley, J., Gentry, T., Ginder-Vogel, M.A., Fienen, M., Mehlhorn, T., Yan, H.,
855 Carroll, S., Pace, M.N., Nyman, J., Luo, J., Gentile, M.E., Fields, M.W., Hickey, R.F., Gu,
856 B.H., Watson, D., Cirpka, O.A., Zhou, J.Z., Fendorf, S., Kitanidis, P.K., Jardine, P.M.
857 and Criddle, C.S. (2006b) Pilot-scale in situ bioremediation of uranium in a highly
858 contaminated aquifer. 2. Reduction of U(VI) and geochemical control of U(VI)
859 bioavailability. *Environ. Sci. Technol.* 40, 3986-3995.

860 Wu, W.M., Carley, J., Luo, J., Ginder-Vogel, M.A., Cardenas, E., Leigh, M.B., Hwang, C.C.,
861 Kelly, S.D., Ruan, C.M., Wu, L.Y., Van Nostrand, J., Gentry, T., Lowe, K., Mehlhorn,
862 T., Carroll, S., Luo, W.S., Fields, M.W., Gu, B.H., Watson, D., Kemner, K.M., Marsh,
863 T., Tiedje, J., Zhou, J.Z., Fendorf, S., Kitanidis, P.K., Jardine, P.M. and Criddle, C.S.
864 (2007) In situ bioreduction of uranium (VI) to submicromolar levels and reoxidation by
865 dissolved oxygen. *Environ. Sci. Technol.* 41, 5716-5723.

866 Yong, P. and Macaskie, L.E. (1995) Enhancement of uranium bioaccumulation by a *Citrobacter*
867 sp. via enzymatically-mediated growth of polycrystalline $\text{NH}_4\text{UO}_2\text{PO}_4$. *J. Chem.*
868 *Technol. Biotechnol.* 63, 101-108.

869 Yung, M.M.C., Ma, J.C., Salemi, M.R., Phinney, B.S., Bowman, G.R. and Jiao, Y.Q. (2014)
870 Shotgun Proteomic Analysis Unveils Survival and Detoxification Strategies by
871 *Caulobacter crescentus* during Exposure to Uranium, Chromium, and Cadmium. J.
872 Proteome Res. 13, 1833-1847.

873 Zabinsky, S.I., Rehr, J.J., Ankudinov, A., Albers, R.C. and Eller, M.J. (1995) Multiple-scattering
874 calculations of X-ray absorption spectra. Phys. Rev. B 52, 2995-3009.

TABLES

Table 1. Orthophosphate liberation rate and Inositol Hexaphosphate (IP₆, Phytate) consumption rate in pH 5.5 reactors amended with 10 mM Phytate (+ P – G – U); 10 mM Phytate and 200 μM U(VI) (+ P – G + U); 10 mM Phytate and 10 mM glycerol (+ P + G – U); or 10 mM Phytate, 10 mM Glycerol, and 200 μM U(VI) (+ P + G + U).

<i>Treatment</i>	<i>d(ΣPO₄³⁻)/dt</i>	<i>d(IP₆)/dt</i>
+ P – G – U	13.5 ± 2.6 mMd ⁻¹	0.92 ± 0.22 mMd ⁻¹
+ P + G – U	20.5 ± 5.1 mMd ⁻¹	2.5 ± 0.58 mMd ⁻¹
+ P – G + U	4.6 ± 0.3 mMd ⁻¹	1.7 ± 0.38 mMd ⁻¹
+ P + G + U	5.6 ± 0.4 mMd ⁻¹	2.9 ± 0.21 mMd ⁻¹

Table 2. Fitting Parameters for U L_{III}-edge EXAFS derived using SIXPACK (Webb, 2005) in soil slurries treated with a (pH 5.5, 200 μ M U), b (pH 5.5, 200 μ M U + 10 mM IP₆), c (pH 5.5, 200 μ M U + 10 mM IP₆ + 10 mM glycerol), d (pH 7.0, 200 μ M U + 10 mM DIC), e (pH 7.0, 200 μ M U + 10 mM DIC + 10 mM IP₆), and f (pH 7.0, 200 μ M U + 10 mM IP₆ + 10 mM glycerol + 10 mM DIC). N represents U-ligand coordination number, R($\text{\AA}$) represents U-ligand distance.

<i>Treatment</i>	<i>Shell</i>	<i>N</i>	<i>R</i> ($\text{\AA}$)	σ^2 ($\text{\AA}^2$)	ΔE_0 (eV)	<i>R factor</i>
A	O _{ax}	2.0	1.78(0.01)	0.005(0.001)	6.21(3.90)	0.0098
	O _{eq}	3.26(1.43)	2.26(0.03)	0.009		
	O _{eq}	1.77(0.64)	2.42(0.03)	0.003		
	Mn/Fe	0.77(0.39)	3.38(0.06)	0.003		
B	O _{ax}	2.0	1.79 (0.01)	0.004(0.001)	10.0(2.13)	0.0159
	O _{eq}	4.04(0.95)	2.34(0.02)	0.009		
	Mn/Fe	0.63(0.23)	3.46(0.02)	0.003		
	P	0.39(0.44)	3.88(0.06)	0.002		
C	O _{ax}	2.0	1.78(0.01)	0.004(0.001)	8.72(2.29)	0.0122
	O _{eq}	3.79(0.93)	2.34(0.02)	0.009		
	Mn/Fe	0.58(0.22)	3.45(0.02)	0.003		
	P	0.55(0.45)	3.89(0.05)	0.002		
D	O _{ax}	2.0	1.78(0.02)	0.006(0.001)	3.99(4.37)	0.0085
	O _{eq}	3.28(1.23)	2.25(0.04)	0.009		
	O _{eq}	1.20(0.46)	2.41(0.04)	0.003		
	C	0.60(0.24)	2.90(0.03)	0.009		
	Mn/Fe	0.24(0.14)	3.43(0.04)	0.003		
E	O _{ax}	2.0	1.77(0.02)	0.003(0.002)	4.70(3.51)	0.0336
	O _{eq}	4.70(1.90)	2.29(0.03)	0.009		
	C	1.59(0.63)	2.94(0.03)	0.009		
	Mn/Fe	0.31(0.35)	3.49(0.06)	0.003		
F	O _{ax}	2.0	1.77(0.01)	0.004(0.001)	7.10(2.55)	0.0145
	O _{eq}	4.91(1.32)	2.29(0.02)	0.009		
	C	0.74(0.32)	2.92(0.03)	0.009		
	Mn/Fe	0.66(0.23)	3.45(0.02)	0.003		

Table 3. Predicted solution equilibrium and solid phase saturation indices using MINEQL+ (Schecher and McAvoy, 2001) in pH 5.5 and 7.0 soil slurries. Solution concentrations are reported in percent of total species. For solid phase species, values reported represent saturation indices for each mineral phase, and numbers in parentheses represent the percentage of uranium precipitated as each respective mineral. Each pH 7.0 treatment was modeled as a closed system (DIC = 10 mM) using the maximum measured inorganic phosphate concentration and initial conditions as input for all other species. A double layer sorption model onto amorphous Fe-oxide (0.41 g/L, 600 m²/g surface area) was included in the calculations. Unless otherwise noted, log K values are as reported in Schecher and McAvoy (2001).

U(VI) Species	Log K	Treatment			
		pH 5.5	pH 5.5	pH 7.0	pH 7.0
		0 mM ΣPO ₄ ³⁻ 200 μM UO ₂ ²⁺	40 mM ΣPO ₄ ³⁻ 200 μM UO ₂ ²⁺	0 mM ΣPO ₄ ³⁻ 200 μM UO ₂ ²⁺	40 mM ΣPO ₄ ³⁻ 200 μM UO ₂ ²⁺
Aqueous					
UO ₂ ²⁺		1.4%	0.0%	0.0%	0.0%
(UO ₂) ₂ OH ₂ ²⁺	-5.62 ^{a,b}	1.2%	0.0%	0.0%	0.0%
UO ₂ OH ⁺	-5.20 ^{a,b}	1.8%	0.0%	0.0%	0.0%
(UO ₂) ₃ (OH) ₅ ⁺	-15.55 ^{a,b}	5.6%	0.0%	0.0%	0.0%
UO ₂ PO ₄ ⁻	13.23 ^b	0.0%	0.0%	0.0%	0.0%
UO ₂ (CO ₃) ⁴⁻	21.84 ^c	0.0%	0.0%	30.5%	0.0%
UO ₂ (CO ₃) ₂ ²⁻	16.81 ^c	0.0%	0.0%	9.2%	0.0%
CaUO ₂ (CO ₃) ₃ ²⁻	27.18 ^d	0.0%	0.0%	46.8%	0.0%
Ca ₂ UO ₂ (CO ₃) ₃	30.7 ^d	0.0%	0.0%	3.7%	0.0%
Fe(wk)OH-UO ₂ (OH) ₂	-6.28 ^e	6.5%	0.0%	0.0%	0.0%
Fe(st)OH-UO ₂ (OH) ₂	-2.57 ^e	11.4%	0.0%	9.2%	0.0%
Solid Phase					
Schoepite	-5.2 ^a	0(71.6% U)	-5.5	-1.816	-5.965
Na-Autunite	47.4	-28.5	0 (100% U)	-29.1	0 (100% U)
Ca-Autunite	27.2 ^a	-30.8	-2.5	-31.5	-3.503

^a (Langmuir, 1997), ^b (Guillamont et al., 2003), ^c (Guillaumont et al. 2003), ^d (Dong and Brooks, 2006), ^e (Waite et al., 1994)

FIGURE CAPTIONS

Figure 1: A) Phytate (IP_6) speciation as a function of pH as calculated by MINEQL+ (Schecher and McAvoy, 2001) using pK_a values reported in Costello et al. (1976). Total phytate concentrations set at 10 mM. Vertical dashed lines represent the experimental conditions of the present study. B) Uranium solubility in the presence of increasing concentrations of phytate at both pH 5.5 (close squares) and 7.0 (open circles) in artificial groundwater equilibrated for 1 week. Initial uranium concentration was 200 μM in all experiments (horizontal dashed line), and error bars represent variation between triplicate experiments and analytical error on duplicate measurements. Labels 1, 2, 3, 4, 5, 6, 7, and 8 represent $[\text{IP}_6]:[\text{U}]$ molar ratios of 1:4, 1:2, 1:1, 3:1, 5:1, 15:1, 25:1 and 50:1.

Figure 2: Evolution of inorganic phosphate (ΣPO_4^{3-}) as a function of time in pH 5.5 (symbols with solid lines) and pH 7.0 (symbols with dashed lines) sediment slurries containing 16 g/L Area 3 Oak Ridge Field Research Center sediments and artificial groundwater amended with: A) 10 mM phytate and 10 mM glycerol (+ P + G – U); 10 mM phytate only (+ P – G – U); and B) 10 mM phytate, 10 mM glycerol, and 200 μM UO_2^{2+} (+ P + G + U); 10 mM phytate and 200 μM UO_2^{2+} (+ P – G + U) and 200 μM UO_2^{2+} only (– P – G + U). All pH 7.0 reactors also received 10 mM dissolved inorganic carbon (DIC) to account for the potential effect of carbonates on uranyl speciation at that pH. Closed symbols represent slurries amended with glycerol and triangles represent slurries without uranium. Error bars include variation between triplicate reactors and the analytical error on duplicate measurements. The short-dashed box delineates the zoomed area depicted in Figure 4B.

Figure 3: Evolution of inositol hexaphosphate (IP_6) and its lower derivatives (IP_x , $x = 2:5$) as a function of time in pH 5.5 (symbols with solid lines) and pH 7.0 (symbols with dashed lines)

slurries containing 16 g/L Area 3 Oak Ridge Field Research Center sediments in artificial groundwater amended with: A) 10 mM phytate (+ P - G - U); B) 10 mM phytate and 10 mM glycerol (+ P + G - U); C) 10 mM phytate, and 200 μM UO_2^{2+} (+ P - G + U); and D) 10 mM phytate, 10 mM glycerol, and 200 μM UO_2^{2+} (+P + G + U). Reactors buffered at pH 7.0 were also amended with 10 mM dissolved inorganic carbon (DIC) to account for the potential effect of carbonates on uranyl speciation at that pH. Error bars include variation between triplicate reactors and analytical error from calibrations.

Figure 4: A) Evolution of dissolved uranium as a function of time in pH 5.5 (symbols with solid lines) and pH 7.0 (symbols with dashed lines) sediment slurries containing 16 g/L Area 3 Oak Ridge Field Research Center sediments and artificial groundwater amended with 10 mM phytate, 10 mM glycerol, and 200 μM UO_2^{2+} (+ P + G + U); 10 mM phytate and 200 μM UO_2^{2+} (+ P - G + U); and 200 μM UO_2^{2+} only (- P - G + U). All pH 7.0 reactors also received 10 mM dissolved inorganic carbon (DIC) to account for the potential effect of carbonates on uranyl speciation at that pH. The short-dashed box delineates the zoomed area depicted in Figure 4B. B) Close up view of the evolution of inorganic phosphate (ΣPO_4^{3-}) (red) and dissolved uranium (black) during the first week of incubation of pH 5.5 soil slurries shown in Figures 2 and 4A; these slurry incubations contained 16 g/L Area 3 Oak Ridge Field Research Center sediments and artificial groundwater amended with 10 mM phytate, 10 mM glycerol, and 200 μM UO_2^{2+} (+ P + G + U) or 10 mM phytate and 200 μM UO_2^{2+} (+ P - G + U). Closed and open symbols represent slurries with and without glycerol, respectively. Error bars represent variation between triplicate reactors and include the analytical error on duplicate measurements.

Figure 5: Solid phase-associated U in: A) pH 5.5; and B) pH 7.0 sediments after 16 days of incubation extracted using a modified sequential extraction technique of Tessier (1979). Bars

943 represent the fraction of U extracted during each individual extraction step relative to total
944 extracted uranium in each treatment. Symbols represent the percent uranium recovered in each
945 treatment. All error bars include the variation between triplicate reactors and duplicate
946 extractions.

947 **Figure 6:** Uranium: A) XANES; B) R-space; and C) k-space diagrams of the L_{III}-edge EXAFS
948 obtained from Area 3 sediments of the Oak Ridge Field Research Center following aerobic
949 incubation in sediment slurries for 16 days at pH 5.5 and 7.0. Treatments included a: pH 5.5, 200
950 μM U; b: pH 5.5, 200 μM U + 10 mM IP_6 ; c: pH 5.5, 200 μM U + 10 mM IP_6 + 10 mM
951 glycerol; d: pH 7.0, 200 μM U; e: pH 7.0, 200 μM U + 10 mM IP_6 ; and f: pH 7.0, 200 μM U + 10
952 mM IP_6 + 10 mM glycerol.

Figure

Figure 1.- Salome et al., 2015

Figure 2.- Salome et al., 2015

Figure 3.- Salome et al., 2015

Figure 4. – Salome et al., 2015

Figure 5.- Salome et al., 2015

Figure 6.- Salome et al., 2015