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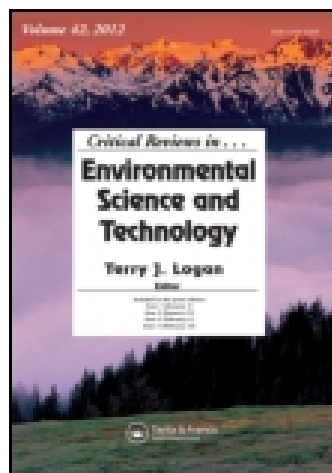


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Reoxidation of Biogenic Reduced Uranium: A Challenge Toward Bioremediation

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Uraninite (UO₂) is the most desirable end product of in situ bioreduction because of its low solubility under reducing conditions. For effective long-term immobilization of uranium (U), there should be no biotic or abiotic reoxidation of the insoluble biogenic U(IV). It is therefore critical to understand the long-term stability of U(IV) under oxic- and nutrient-limited conditions at U-contaminated subsurface sites. It has now been established that following in situ bioremediation of U(VI) via nutrient addition in the subsurface, a range of physical, chemical, and biological factors control the rate and extent of long-term stability of U(IV). Some of these factors are tied to site specific conditions including existence of oxidants such as Fe(III)(hydr)oxides, Mn(IV) oxides, oxygen, and nitrate; the presence of organic carbon and the reduced forms of U (e.g.,

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mononuclear U(IV) or nanometer-sized uraninite particles); and the carbonate concentration and pH of groundwater. This review analyzes the contribution of these factors in controlling U(IV)-reoxidation, and highlights the competition among U(IV) and other electron acceptors and possible mechanisms of reoxidation of various forms of U(IV).

KEY WORDS: biogenic UO_2 , Fe(III)-(hydr)oxides, humics, Mn(IV) oxides, siderophores

1. INTRODUCTION

The most oxidized form of uranium (U), U(VI), tends to be soluble and may exist as different aqueous species depending on the alkalinity, pH, and presence of complexants e.g., UO_2^{2+} , UO_2OH^+ , $\text{UO}_2(\text{OH})_2$, $\text{UO}_3 \cdot 3\text{H}_2\text{O}$, $\text{UO}_2(\text{CO}_3)_2^{2-}$, $\text{UO}_2(\text{CO}_3)_3^{4-}$, and $\text{Ca}_2\text{UO}_2(\text{CO}_3)_3$ (Guillaumont et al., 2003; Dong and Brooks, 2006). These highly mobile U(VI) species present in the subsurface are considered to cause severe ecological and public health hazards due to their high toxicity and long half-lives (Kazy et al., 2009). Reductive precipitation of soluble U(VI) species to less soluble and more stable U(IV) (UO_2 , uraninite) is considered an attractive approach to remediate U-contaminated sediments and groundwater (Brodie et al., 2006; Akob et al., 2008). U(VI) reduction can be catalyzed by microbial processes involving anaerobic dissimilatory metal reducing bacteria (DMRB) present in the subsurface. Typically, organic substrates (e.g., ethanol, lactate, acetate, oleate) are utilized as electron donors to drive biological U(VI) reduction. Laboratory studies and field experiments show that U(VI) reduction occurs after nitrate depletion and during iron or sulfate-reducing conditions (Lovley and Phillips, 1992; Abdelouas et al., 2000; Finneran et al., 2002; Senko et al., 2002; Anderson et al., 2003; Sani et al., 2004; Sani et al., 2005; Wall and Krumholz, 2006; Sivaswamy et al., 2011).

Under reducing conditions, these biologically and chemically reduced U species are considered stable and less mobile. There are at least three types of biogenic U(IV) found in the literature: namely uraninite, nanoparticulate uraninite, and mononuclear U(IV) phases. The former two are more crystalline forms while mononuclear U(IV) is more amorphous (Fletcher et al., 2010; Bernier-Latmani et al., 2010; Boyanov et al., 2011). Evidence indicates that these reduced U forms, including crystalline uraninite, are very susceptible to reoxidation (Beyenal et al., 2004; Moon et al., 2009; Spycher et al., 2011, and references therein), posing challenges to long-term stability of U(IV) in remediated sites. This is mainly due to groundwater influxes continuously introducing U(IV)-oxidizing species (e.g., O_2 , NO_3^- , and NO_2^-). Reoxidation of biogenic U(IV) by Fe(III) and Mn(IV) (which are abundant at U-contaminated sites, even under reducing conditions) has also been shown

in the presence of metal-reducing bacterial activity under electron donor-limited conditions (Fredrickson et al., 2002; Sani et al., 2005; Wan et al., 2005; Zhengji, 2010). Therefore, it is vital to identify and characterize the processes and mechanisms involved in the reoxidation of biogenic U(IV) which control its stability and thus mobility in U-contaminated groundwater and sediments. This review focuses on the current status of knowledge about the reoxidation of biogenic U(IV).

2. REOXIDATION OF BIOGENIC U(IV)

It is important to understand the stability of U(IV) and the rate and extent of its reoxidation when nutrient injection is stopped and oxic conditions return. U(IV) has been shown to oxidize rapidly in the presence of oxygen (Gu et al., 2005; Zhou and Gu, 2005; Moon et al., 2007; Moon et al., 2009; Komlos et al., 2008a; Komlos et al., 2008b), which could potentially remobilize U that was immobilized during biostimulation. U(IV) can also be oxidized under anoxic conditions by denitrification byproducts (Senko et al., 2002), Fe(III) (Sani et al., 2004; Sani et al., 2005; Ginder-Vogel et al., 2006; Ginder-Vogel et al., 2010; Spycher et al., 2011) and Mn(IV) oxides (Fredrickson et al., 2002). Increased bicarbonate concentrations due to microbial respiration have also been shown to increase the favorability of Fe(III)-dependent U(IV) oxidation to U(VI) (Wan et al., 2005; Ginder-Vogel et al., 2006; Spycher et al., 2011). Several other factors involving humic substances (Gu et al., 2005), siderophores (Frazier et al., 2005), and pH (Casas et al., 1998; Ulrich et al., 2008; Ulrich et al., 2009) have also shown to play significant roles in U(IV) oxidation and remobilization. Known mediators and oxidants involved in U(IV) reoxidation are summarized in Table 1 and are discussed subsequently in detail.

2.1 Influence of Fe(III)(hydr)oxides

The geochemistry of anaerobic soil and sediment environments, as well as the solubility of various inorganic contaminants, including U, are strongly controlled by the microbially mediated reduction of Fe(III)-(hydr)oxides (Fredrickson and Gorby, 1996; Abdelouas et al., 1998; Fredrickson et al., 2000; Wielinga et al., 2000; Zachara et al., 2001; Sani et al., 2004). For example, iron [Fe(III/II)] can serve as a reductant or oxidant of U, depending on the specific geochemical conditions (Ginder-Vogel et al., 2006; Ginder-Vogel et al., 2010), and can even switch between being an oxidant and reductant during the course of a single incubation (Wan et al., 2005). A comparison of the reduction potentials of U(VI/IV) couples with various terminal electron acceptors is shown in Figure 1. The redox potentials of Fe(III/II) and U(VI/IV) are comparable, thus the specific species of either element along with the chemical gradients dictate which serve as the oxidant and reductant.

TABLE 1. Reoxidation of reduced U in presence of various mediators and oxidants investigated in the last decade

Microbial strain	Mediators/oxidants for U(IV)	Electron donor for U(VI) reduction	U(IV) oxidation rates ($\mu\text{M}/\text{day}$)	Reference
Fe(III)-reducing bacteria	Fe(III) oxides	Complex organic compounds	ND	Nevin and Lovley (2000)
Mixed subsurface cultures	NO_3^- , NO_2^- , N_2O , NO	Acetate	0.2 to 3.32	Senko et al. (2002)
Mixed subsurface cultures (sediments)	NO_3^- , NO_2^- , N_2O , NO	^{14}C -acetate	1.33 ^a	Finneran et al. (2002)
<i>Geobacter metallireducens</i>	NO_3^- , NO_2^- , N_2O , NO	^{14}C -acetate	138	Finneran et al. (2002)
<i>Geobacter metallireducens</i>	NO_3^- , NO_2^- , N_2O , NO, and Fe(III)	^{14}C -acetate	300	Finneran et al. (2002)
<i>Shewanella putrefaciens</i> strain CN32	β - MnO_2 and Mn_2O_3	Complex organic compounds	260 and 360	Fredrickson et al. (2002)
<i>Desulfohalobium desulfuricans</i> G20	Ferrihydrite, goethite, hematite, & FeCl_3	Lactate	1.76, 2.59, 3.53, and 78 ^b	Sani et al. (2005)
<i>Thiobacillus denitrificans</i>	NO_3^-	Thiosulfate	2.86, 2.47 ^c , and 0.003 ^d	Beller (2005)
<i>Desulfohalobium vulgaris</i>	NO_2^- and Iron(III) oxyhydroxide	Lactate	10 to 130 ^b	Senko et al. (2005)
<i>Shewanella putrefaciens</i> CN32	pH (4–9) and Fe(III)	Lactate	0.126 to 1.55	Zhong et al. (2005)
Mixed subsurface cultures	Carbonate	Lactate	0.01	Wan et al. (2005)
<i>Shewanella putrefaciens</i> CN32	Humic substances and O_2	Lactate	140 to 200	Gu et al. (2005)

<i>Shevuanella putrefaciens</i> CN32 Mixed subsurface cultures	Ferrihydrite, goethite, and hematite O ₂	Lactate	0.70, 0.50, and 0.38 ^b 0.033	Ginder-Vogel et al. (2006) Wu et al. (2007)
Mixed cultures and <i>Geobacter metallireducens</i> <i>Bacillus</i> sp. SG-1	NO ₃ ⁻ and O ₂	Ethanol		
<i>Shevuanella oneidensis</i> MR-1. Mixed cultures and <i>Geobacter metallireducens</i> <i>Geobacteraceae</i> population	MnO ₂ pH	Acetate	21.8 and 7.5	Moon et al. (2007)
<i>Shevuanella oneidensis</i> MR-1	Oxic water (O ₂)	Organic compounds Lactate	0.13 to 3.67 0.07 ^f to 3707 ^f	Chinni et al. (2008) Ulrich et al. (2008)
	Organic carbon supply	Acetate	66 ^e	Komlos et al. (2008)
	Oxygen (O ₂) and carbonate concentrations	Lactate	0.08	Wan et al. (2008)
A mixed culture of sulfate reducing bacteria	Goethite and hematite	Lactic acid	830 ^{f,g} and 210 ^{f,g} ; 9.9 ^h to 2688 ^h ; 6419 ^{f,i} and 6847 ^{f,i}	Ulrich et al. (2009)
		Complex organic compounds	0.013 and 0.04	Zhengji (2010)

Note. ND = not determined (U data are not present).

^aUraninite (Alfa Products, Danvers, MA).

^bAbiotic reoxidation.

^cSynthetic U(IV) oxide.

^dAggregated grains of U(IV) oxide.

^eOxic water was introduced into a column.

^fBased on mass of bio-UO₂ solids.

^gAnoxic conditions.

^hModerately oxidizing conditions.

ⁱStrongly oxidizing conditions.

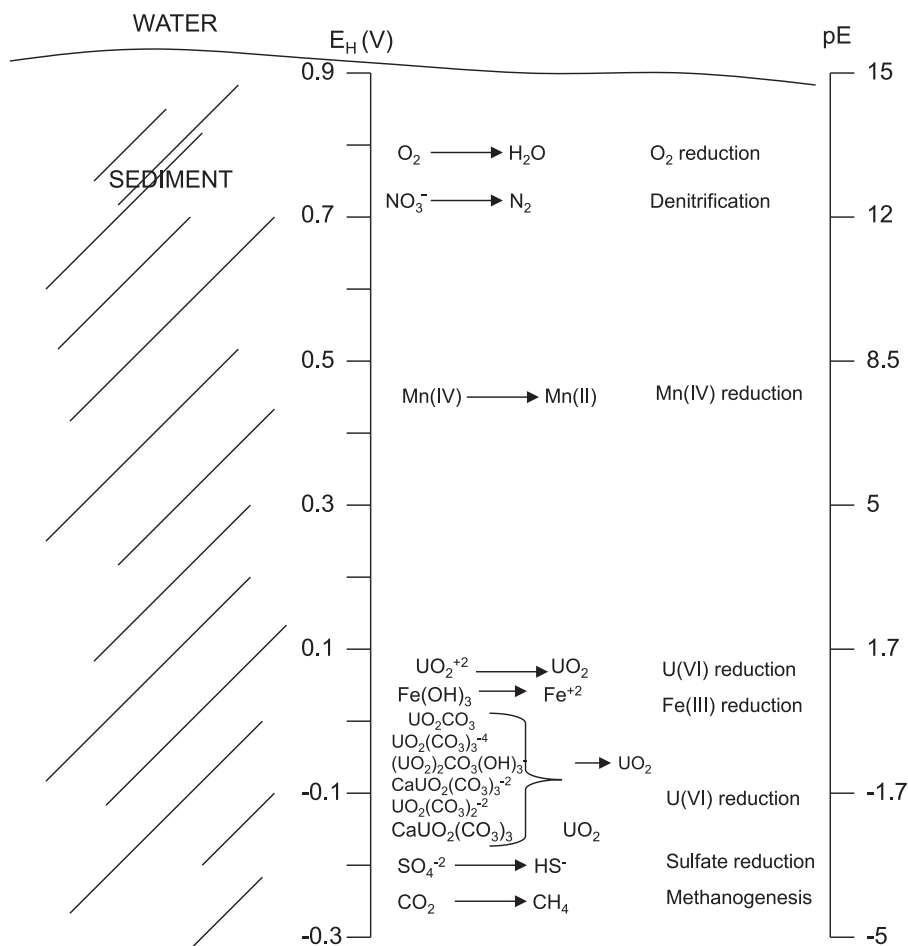


FIGURE 1. Various terminal electron acceptors with corresponding redox potentials.

Perturbations in aqueous chemistry may also result in oscillations between thermodynamic viability and nonviability of $U(IV)$ oxidation by $Fe(III)$ (Ginder-Vogel et al., 2006). Depending on the specific species of U , the $U(VI/IV)$ redox potentials can change more than 0.2 V, with UO_2^{2+} leading to the highest potential and $Ca_2UO_2(CO_3)_3$ the lowest (Figure 1). It has also been suggested that $U(IV)$ is more susceptible to oxidation when $U(VI)$ - $CaCO_3$ complexes dominate (Stewart et al., 2007). At the Old Rifle Uranium Mill Tailings Remedial Action Site, however, U remobilization was observed following transition from $Fe(III)$ -reducing to sulfate-reducing conditions (Anderson et al., 2003). In these experiments, $U(VI)$ and $Fe(III)$ were reduced simultaneously. A loss of sulfate from the groundwater was also observed during acetate injection with an accumulation of sulfide. During this biostimulation event, the composition of the microbial community also changed (Anderson et al., 2003). Data suggest that iron-reducing bacteria (FeRB)

initially dominated the microbial community. As soon as readily bioavailable Fe(III) was reduced, the system shifted to sulfate-reducing conditions, which then controlled the stability of the bioreduced U. In addition, it has been shown using column experiments with natural materials that sulfate reduction can commence even though significant bioavailable Fe(III), a more energetically favorable electron acceptor, remains (Komlos et al., 2008a).

Sulfate reducing bacteria (SRB) have been shown to reduce U(VI) in the absence of Fe(III)(hydr)oxides under non-growth conditions (Ganesh et al., 1997; Ganesh et al., 1999; Lovley and Phillips, 1992; Lovley et al., 1993a; Lovley et al., 1993b; Spear et al., 1999; Spear et al., 2000; Payne et al., 2002) and in the presence of Fe(III)(hydr)oxides under growth conditions (Sani et al., 2004; Sani et al., 2005; Zhengji, 2010). It has also been shown that U(IV) produced by SRB (uraninite crystals of 3–5 nm diameter) can be reoxidized in the presence of SRB and Fe(III)(hydr)oxides (e.g., hematite, goethite, and ferrihydrite) under electron donor-limited conditions (Sani et al., 2004; 2005). Sulfide production by SRB activity is known to decrease Fe(II) product concentrations via Fe-sulfide precipitation and/or formation of strong Fe(II) sulfide complexes, which can drive U(IV) oxidation according to the equation, $\text{U(IV)} + 2\text{Fe(III)} \leftrightarrow \text{U(VI)} + 2\text{Fe(II)}$. In addition to the activity of Fe(II), U(IV) reoxidation is expected to depend on the rates of sulfide oxidation by Fe(III) (Spycher et al., 2011). A laboratory and field study, however, showed that Fe-sulfide precipitates formed during bioreduction (e.g., mackinawite $\text{FeS}_{0.9}$) protected oxidation of UO_2 by scavenging the dissolved oxygen (Dullies et al., 2010).

Thermodynamic studies confirmed the experimental observations of U(IV) oxidation by Fe(III)(hydr)oxides under specific conditions (e.g., Wan et al., 2005; Ginder-Vogel et al., 2006). However, a better understanding of the mechanisms driving U(IV) oxidation is needed to accurately predict rates, stability, and permanence of anaerobic microbial metal transformations that appear to be very important in a variety of subsurface environments. One of the many challenges in this area of research is predicting the oxidation/reduction products in natural environments that exhibit complex and coupled biogeochemical interactions. For example, the formation of highly stable $\text{Ca-UO}_2^{2+}\text{-CO}_3$ complexes [e.g., $\text{Ca}_2\text{UO}_2(\text{CO}_3)_3$] resulting from accumulation of carbonate from microbial respiration and calcite dissolution can lead to U(IV) oxidation under “reducing” conditions (Wan et al., 2005). Fe(III)(hydr)oxides and Mn oxides present in this system were the only likely electron acceptors available for U(IV) reoxidation in these experiments.

It has now been established that Fe(III)-(hydr)oxides have the potential to reoxidize (and thus remobilize) U(IV). For example, U could act as an electron shuttle between *Geobacter* cells and ferrihydrite, suggesting the possibility of Fe(III)-hydroxide-mediated U(IV) oxidation in natural systems (Nevin and Lovley, 2000). The addition of Fe(II) to a *Geobacter* cell suspension also stimulated reoxidation of biogenic U(IV) (Finneran et al., 2002). It was speculated that the freshly precipitated Fe(III)-hydroxides during the

biological Fe(II) oxidation coupled to nitrate reduction reoxidized U(IV). Ginder-Vogel et al. (2006) demonstrated that biogenic U(IV) oxidation by ferrihydrite was thermodynamically favorable under conditions typical of many contaminated sites. Furthermore, the extent of biogenic U(IV) oxidation by ferrihydrite increased with increasing bicarbonate concentration. Experimental data (Sani et al., 2004; Sani et al., 2005; Zhengji, 2010) also support the hypothesis that Fe(III) can serve as an effective electron acceptor for biogenic U(IV) even under strongly reducing conditions. Thus, the availability of residual Fe(III) leads to the reoxidation of this biogenic U(IV), thereby significantly affecting U speciation and partitioning between aqueous and solid phases. Most Fe(III) occurs as an insoluble mineral surface which can change kinetics and ultimately the extent of reaction. Further fundamental research is needed to characterize and model these important reactions to elucidate U geochemical speciation in the subsurface environment. The studies of Sani et al. (2004; 2005) have also shown the potential for the reoxidation of biogenic U(IV) with Fe(III)-(hydr)oxides in the presence of SRB in field-relevant scenarios. On the other hand, these studies showed that in an abiotic system, soluble Fe(III) oxidized uraninite nanocrystals, whereas, under the same conditions, a crystalline form of Fe(III) oxide (hematite) did not. In contrast to the abiotic system, hematite oxidized U(IV) in the presence of SRB activity. These findings suggest that SRB activity somehow activates the reaction of Fe(III)-(hydr)oxides with nanocrystalline uraninite.

Many of the published experimental and modeling results (Nevin and Lovley, 2000; Finneran et al., 2002; Sani et al., 2004; Sani et al., 2005; Senko et al., 2005; Wan et al., 2005; Ginder-Vogel et al., 2006; Zhengji, 2010; Spycher et al., 2011) clearly demonstrate the oxidation of biogenic U(IV) (including uraninite nanocrystals) through Fe(III)-(hydr)oxides. These findings suggest that complete reduction of all Fe(III) at any particular site would be required to stabilize U in situ. Mechanisms for biogenic U(IV) oxidation by Fe(III)-(hydr)oxides are still not clear. It can be speculated that the oxidation of U(IV) by Fe(III)-(hydr)oxides will likely occur through the direct contact of biogenic U(IV) with (a) Fe(III) minerals; (b) Fe(III)-chelating compounds produced by SRB that have solubilized Fe(III) from Fe(III)-(hydr)oxide solids; or (c) electron shuttle compounds that accept electrons from U(IV) and transfer them to Fe(III)-(hydr)oxides. Because the long-term stability of U(IV) and its reoxidation rate in a given field setting would depend on the mechanisms of the U reoxidation, it is vital to understand and quantify the governing processes of U reoxidation.

2.2 Influence of Manganese Oxides

Manganese oxides are prevalent at U-contaminated sites and are known to influence the biogeochemical behavior of U (Chinni et al., 2008). For example, experiments were conducted using various U(VI) forms and concentrations

during Mn oxide biogenesis, which showed a strong uptake of U (via sorption and incorporation) by Mn oxide biominerals, leading to more stable U(VI) sequestration which may be suitable for remediation purposes (Webb et al., 2006). On the other hand, Veeramani et al. (2009) observed that the equilibrium solubility of Mn(II)-reacted biogenic uraninite was halved and its dissolution rate was up to 38-fold lower relative to unreacted uraninite. This demonstrates the stabilizing effect of structural Mn(II) incorporation by biogenic uraninite. A study by Fredrickson et al. (2002) also showed that the overall rates of microbial U(VI) reduction using *Shewanella putrefaciens* strain CN32 decreased in the presence of Mn(III/IV) oxides (β -MnO₂, Mn₂O₃, and K₄Mn₁₄O₂₇·8H₂O). One of the reasons for this was that the U functioned as an electron shuttle between DMRB and Mn(III/IV) oxides, which caused a decrease in the overall extent of U(VI) reduction due to abiotic reoxidation of U(IV) to U(VI) by Mn(III/IV) oxides. It has been shown that U(IV) oxidation by β -MnO_{2(s)} is thermodynamically favorable over a wide range of reactant and product concentrations at neutral pH in bicarbonate-based solutions (Fredrickson et al., 2000; Fredrickson et al., 2002). In another experiment involving abiotic oxidation of biogenic uraninite by β -MnO_{2(s)}, the ratio of Mn(II) to U(VI) was observed to be 0.91 (Liu et al., 2002), consistent with the expected stoichiometry for the reaction coupling the reduction of β -MnO_{2(s)} to the oxidation of UO_{2.09(s)}. In an attempt to model U(IV) reoxidation by β -MnO_{2(s)}, Liu et al. (2002) calculated the reaction order of the initial biogenic uraninite oxidation coupled to β -MnO_{2(s)} reduction as 0.47 ± 0.04 . However, they could not explain the discrepancies between the predicted (0.47) and observed (0.91) results based on their model, likely due to the fact that the availability of U(IV) for oxidation by β -MnO_{2(s)} changed with time. Based on transmission electron microscopic (TEM) observations, the distribution and surface properties of the biogenic UO_{2(s)} changed during U(VI) reduction in the presence of β -MnO_{2(s)}. These changes have probably affected mass transfer, crystallization, localization, and aggregation of U(IV), and thus contact with microorganisms and β -MnO_{2(s)} particles. These factors were neither well understood nor easily quantified. It was also unclear whether U(IV) was oxidized by β -MnO_{2(s)} in the form of individual ions, disordered precipitate clusters, crystallites, or crystallite aggregates.

The effects of different concentrations of U and Mn on the coupled biogeochemical oxidation-reduction reactions of U and Mn were also investigated (Chinni et al., 2008). Experiments were conducted using spores of Mn(II)-oxidizing *Bacillus* sp. strain SG-1. The results showed that U(IV) was oxidized 30–50 times more in the presence of Mn oxides compared to Mn oxide-free controls. As a consequence of this U(IV) reoxidation, Mn oxides were reduced to soluble Mn(II) that were likely reoxidized back again by *Bacillus* SG-1 spores. *Bacillus* SG-1 spores did not directly oxidize U(IV), but U(IV) oxidation proceeded rapidly in the presence of Mn(II) concentrations as low as $<5 \mu\text{M}$. The rates of U(IV) oxidation were equivalent to the rates

of Mn oxide reduction, and depended on the initial concentrations of U(IV), dissolved Mn(II), or Mn(IV) oxides. U(VI) or U(IV) decreased the Mn(II) oxidation rate by inhibiting the Mn(II)-oxidizing enzyme or decreasing the available Mn(II). These results suggest that to predict the fate, transport, and stability of U(IV) in the subsurface, the roles of Mn(II)-oxidizing bacteria should be considered.

2.3 Influence of Nitrate and Dissolved Oxygen

Several subsurface microorganisms are capable of reducing nitrate and U(VI) (Lovley et al., 1993a). It has been shown that U(VI) reduction by *Desulfovibrio desulfuricans* (ATCC 29577) is slightly inhibited by the presence of 190 mM nitrate but not by nitrate concentrations lower than 95 mM (Ganesh et al., 1999). Laboratory experiments involving heat-inactivated sediment slurries have shown that the intermediates of dissimilatory nitrate reduction (NO_2^- , N_2O , and NO) could oxidize U(IV) (Table 1 in Senko et al., 2002). In a series of push-pull experiments, the impact of electron donor and terminal electron acceptor amendments on the fate of U(VI) was determined in anaerobic sediments from a landfill leachate (Norman, OK) that had not been previously contaminated with U(VI) (Senko et al., 2002). Field experiments and laboratory incubations revealed the potential for in situ U(VI) immobilization through concerted microbiological and geochemical processes. Results also showed that nitrate-reducing activity led to the mobilization of previously immobilized U due to oxidation of U(IV) by dissimilatory nitrate reduction intermediates. In a separate experiment involving the addition of dissimilatory nitrate reduction intermediates to the U(IV), rapid reoxidation of U took place. Gibbs free energy calculation of reactions involving dissimilatory nitrate and U(VI) reduction revealed that U(IV) oxidation by N_2 and NO_3^- was less favorable than by NO_2^- , N_2O , and NO (Senko et al., 2002).

Nitrate grown cells of *Geobacter metallireducens* were also shown to carry out nitrate-dependent UBr_4 (a soluble form of U(IV)) oxidation (Finneran et al., 2002). These findings indicate that in situ subsurface U(VI) immobilization can be expected to take place under anaerobic conditions, but the permanence of the approach could be impaired by dissimilatory nitrate reduction intermediates (especially NO_2^- , N_2O , and NO) that have the ability to oxidize and thereby mobilize previously reduced U. *Thiobacillus denitrificans*, an obligate chemolithoautotrophic bacterium, is known for its ability to couple the oxidation of various inorganic sulfur electron donors (e.g., less soluble minerals such as pyrite and FeS) with nitrate as an electron acceptor. Under anaerobic nitrate-reducing conditions at circumneutral pH, *T. denitrificans* also oxidized synthetic and biogenic U(IV) (Beller, 2005). U(IV) oxidation in these incubations only accounted for <2% of nitrate consumption and ceased while nitrate was still available. These results suggest

that that *T. denitrificans* did not couple U(IV) oxidation and nitrate consumption for energy conservation.

U(IV) oxidation accounted for a small portion (about 10%) of nitrate consumption during nitrate-dependent U(IV) oxidation by the heterotrophic *G. metallireducens* in cell suspension experiments (Finneran et al., 2002). The exact nature of the nitrate dependence on U(IV) oxidation is yet to be determined. For example, does nitrate-dependent U(IV) oxidation involve electron-carrier proteins (e.g., cytochromes)? If yes, what are those electron-carrier proteins, and are those proteins present in all DMRB, or do proteins involved in nitrate reduction have any direct role(s) in U(IV) oxidation? In the case of *T. denitrificans*, however, two membrane-associated diheme *c*-type cytochromes, putatively *c₄* and *c₅*, were shown to play major roles in nitrate-dependent U(IV) oxidation (Beller et al., 2009). Mutations in the genes coding for these two cytochromes, resulted in about 50% decrease in U(IV) oxidation activity and complementation of genes in mutants restored activities similar to those of the wild-type.

Recently *Acidovorax ebreus* TPSY and *Pseudogulbenkiania* sp. strain 2002 were shown to use U(IV) as an electron donor for the reduction of nitrate under non-growth conditions (Weber et al., 2011). Many nitrate-reducing bacteria like *Geothrix fermentans* H5, *Pseudomonas* sp. PK, and *Magnetospirillum* sp. VDY were also shown to oxidize U(IV) in the presence of nitrate. Furthermore, the impact of different geochemical conditions on the composition of the nitrate-reducing microbial community structure has also been recently examined (Spain and Krumholz, 2011). Identification of these bacteria expanded our knowledge about the existing taxonomic diversity of nitrate-dependent U(IV) oxidation. The presence of nitrate-dependent U(IV)-oxidizing bacteria in diverse environments suggests that these bacteria are ubiquitous in the ecosystem and are not necessarily dependent on U exposure (Weber et al., 2011). This knowledge poses a challenge to the efficacy of long-term reductive immobilization of U as U(IV) in subsurface environments where nitrate is present.

Oxygen can oxidize U(IV) rapidly (e.g., with a reoxidation rate of 66 $\mu\text{M/day}$; Komlos et al., 2008b; Table 1). The U.S. Environmental Protection Agency maximum contaminant limit (MCL) of U in drinking water is reported to be 0.126 μM . A study observed U(IV) levels to be stable at $\leq 0.126 \mu\text{M}$ under anaerobic, quiescent conditions at pH values near 6 over a period of four years; however, with the injection of 0.25–0.34 mM dissolved oxygen (DO) and discontinued sulfite addition, the U levels increased back, up to 1.87 μM , within a period of 60 days (Wu et al., 2007). This suggests the need to develop both additional methods for DO removal from water and more efficient and cost-effective strategies for the creation of solid-phase forms of U(IV) that resist oxidation. Column experiments showed that both oxygen and nitrate reduction intermediates remobilized (88% and 97%, respectively) the majority of the U precipitated during bioreduction within

54 days (Moon et al., 2007). It was very interesting to observe that oxygen-dependent U oxidation was less than nitrate-dependent oxidation, likely due to the reactivity of oxygen with other reduced compounds present in the column. Recently it was shown that the oxidative dissolution of biogenic uraninite when deployed in situ into wells under variable oxic conditions greatly depended on the aquifer permeability, native microbes and groundwater geochemistry (Campbell et al., 2011a). The rates of U dissolution were observed to be 50–100 times lower under aquifer conditions compared to the laboratory studies, and the presence of biomass in the biogenic uraninite deployed wells in the aquifer was observed to further retard the dissolution in the field. This retardation in uraninite oxidation rates under field conditions was likely due to diffusional constraints and surface passivation by groundwater solutes (Campbell et al., 2011b). Taken together, published results suggest that the presence of oxidants (e.g., NO_2^- , N_2O , NO , and O_2), aquifer permeability, indigenous microbes, and groundwater geochemistry will likely severely affect the fate, transport, and stability of reduced U during and after in situ U bioremediation.

2.4 Influence of Carbonates and pH

Uranium behavior in situ is also affected by the presence of carbonates and variations in pH. A study involving long-term (17 months) sediment column experiments, where columns were packed with U-contaminated sediments infused with sufficient organic carbon (OC) to maintain reducing conditions, showed that U was reduced during the first 80 days. However, following this (100–500 days), U remobilization was observed despite the presence of a microbial community belonging mainly to *Geobacteraceae* capable of reducing U(VI) (Wan et al., 2005). Tokunaga et al. (2008) also observed an increase in U(IV) reoxidation due to the formation of strong U(VI) carbonate complexes that were generated from carbonate produced during microbial degradation.

A comparative study of the application of magnetite nanoparticles (nano- Fe_3O_4) and zerovalent iron nanoparticles (nano- $\text{Fe}(0)$) for U removal from carbonate rich waters was conducted by Crane et al. (2011). Both nano- $\text{Fe}(0)$ and nano- Fe_3O_4 systems were effective in removing U initially (98% and 20%, respectively) but after one week rebound of U(VI) was observed mainly due the presence of carbonates. Therefore, the presence of high concentrations of bicarbonate can promote the formation of stable uranyl carbonate complexes as well as reoxidation of the biogenic U(IV) by site specific unreduced Fe(III) and Mn(IV) as described above. The rebound in U(VI) concentrations were also observed upon transitioning to sulfate-reducing conditions (e.g., Fang et al., 2009; Yabusaki et al., 2011; Williams et al., 2011). This was mainly due to desorption promoted by significant increases in bicarbonate resulting from sulfate reducing activity, increases

in pH, alkalinity, and calcium, and lower U(VI) bioreduction in the system. Yabusaki et al. (2011) concluded that when acetate concentrations were tripled in excess of sulfate reducing bacteria (SRB) demand, more acetate became available for iron reducing bacteria (FeRB), which then decreased U(VI) concentrations.

The influence of OC supply rates on mobility of biogenic U(IV) in sediments was also evaluated (Wan et al., 2008). The study showed that the OC supply rate was a key factor controlling the mobilization of U(IV) when reactive Fe(III) (in the form of amorphous Fe(III) or ferrihydrite) was still present in the sediment to serve as the terminal electron acceptor for the U(IV) oxidation. Excessive OC supply rates resulted in increased bicarbonate concentration levels leading to the formation of stable uranyl carbonate complexes, promoting the reoxidation of U(IV) and/or desorption of unreacted U(VI). Although lower OC supply rates prevented carbonate driven U(IV) reoxidation within the time period of the experiments, it could not sustain reducing conditions for longer times. Molecular analyses of total microbial activity demonstrated a positive correlation with OC supply. Analyses of *Geobacteraceae* activity using RT-qPCR of 16S rRNA indicated a continued activity even when the effluent Fe(II) became undetectable (Wan et al., 2008).

Casas et al. (1998) carried out natural UO₂ (samples from Cigar Lake, Jachymov, and Oklo) and synthetic UO₂ solubility studies as a function of pH and under nominally reducing conditions in a solution containing 0.008 mol L⁻¹ perchlorate and 1 mol L⁻¹ chloride. Differences in solubility of natural and synthetic UO₂ samples were likely due to the presence of carbonate in their experiments, whereas the differences in solubility observed among the natural samples could be correlated to radiation effects at atomic scale. In a process of extracting a U-contaminated soil with bicarbonate at varying concentrations (0–1 M), pHs, and redox conditions, Zhou and Gu (2005) found at least three different U forms of uranium compounds: (a) uranium phosphate minerals, (b) reduced U(IV) phases, and (c) U(VI) complexed with soil organic matter. They demonstrated oxidative remobilization of freshly prepared and one-year aged biogenic UO₂ in 0.1 mol/L NaNO₃ electrolyte with variable pH and Fe(II) concentrations. Interestingly, biogenic UO₂(s) was released into the aqueous phase with the highest rate and extent at both pH 4 and 9, while the U remobilization was the lowest at circumneutral pH. Ex situ flow through experiments showed faster U mobilization than observed in column oxidation studies (Sharp et al., 2011). Ulrich et al. (2008; 2009) quantified and compared intrinsic solubility and dissolution rate constants of biogenic UO₂ and synthetic UO₂ by taking molecular-scale structures into account. The dissolution rates of biogenic and synthetic UO₂ solids, which were determined as a function of pH and dissolved inorganic carbon in continuous-flow experiments under anoxic conditions, were lowest at near neutral pH and increased with decreasing pH. It is therefore possible that

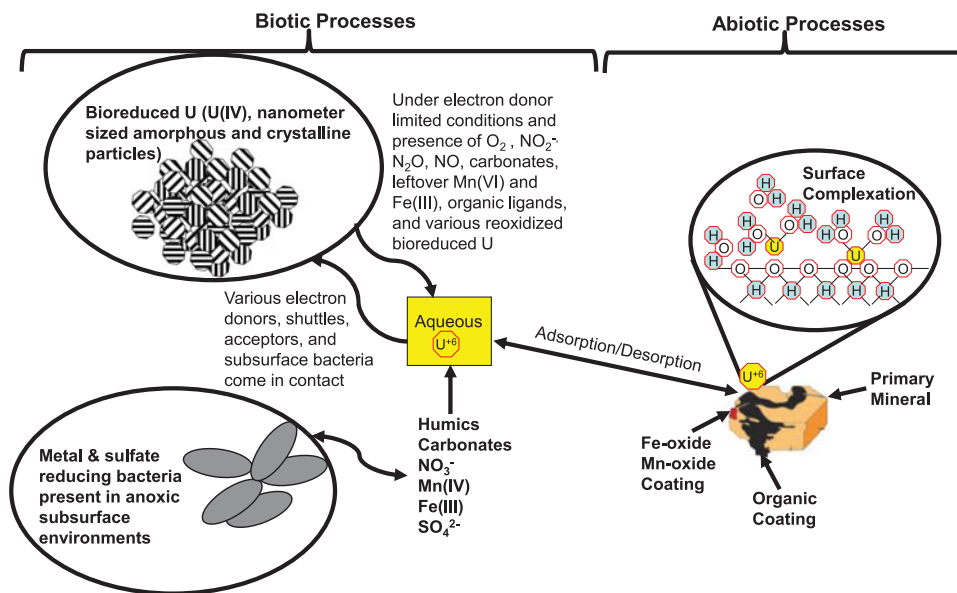


FIGURE 2. Summary of various factors involved in reoxidation and nanometer sized particles of bioreduced uranium in the subsurface (modified from Palmisano and Hazen, 2003).

the rate and extent of biogenic UO_2 oxidative remobilization may be controlled by manipulating subsurface biogeochemical conditions such as pH and carbonate concentrations. These various factors involved in reoxidation of biogenic U(IV) in the subsurface are summarized in Figure 2.

2.5 Influence of Humic Substances and Organic Ligands

Humic substances (e.g., humins, humic acids, fulvic acids) occur naturally in soil and groundwater, and play important roles in the conversion of U(VI) to U(IV) and vice versa. It has recently been suggested that much lower solubility of U(VI) was observed after treatment with humic acids (Wan et al., 2011), which showed humic acids were beneficial in U remediation. Also in a field pull-push test, humic materials enhanced the U(VI) reduction rate up to 10 fold. During the same experiment, however, the oxidation of U(IV) was increased with an oxidation half-life of less than 5 min (Gu et al., 2005) upon exposure to oxygen. On the other hand, the reoxidation of U(IV) in water or in 0.03 M NaHCO_3 solution (humic-free) had an extended half-life of ~40 min. It was likely due to the formation of U(IV) complexes with humic substances during the bioreduction process that would have prevented the precipitation of the U(IV) into a solid phase and thus increased the U(IV) reoxidation rate. When humic substances were added separately to reduced U(IV) solids that were formed in the absence of humic substances, the oxidation rates of U(IV) were comparable to those with added water or 0.03 M

NaHCO₃ solution. This was likely due to the existence of reduced U(IV) and the humic substances in different phases, where the oxidation rate of U(IV) was limited by the solubilization of the U(IV) solids in the presence of oxygen and carbonates. This complexation reaction presumably occurred through the coordination of reduced U(IV) and chelating functional groups of humic substances such as carboxyls, hydroxyls, and ketones. Direct evidence of the formation of soluble U(IV)-citrate complexes have also been provided (Francis et al., 2002). The formation of U(IV) complexes in the presence of organic ligands such as oxalate, triton, and citrate, which contain carboxyl and hydroxyl chelating groups such as those found in humic substances, has also been reported (Ganesh et al., 1997). From a thermodynamic standpoint, these U(IV) complexes will likely be more susceptible to oxidation. Recently our group also showed that the presence of chelating agents (e.g., citrate, EDTA, and NTA; Stewart et al. 2013) stimulated the oxidation of UO₂(s) through the formation of stable U(VI) complexes. Luo and Gu (2011) demonstrated the effective mobilization of U, Fe, and Al by complexing organic ligands in bioreduced sediments, where EDTA was observed to be more effective than citrate in dissolving these minerals. In summary, humic substances and organic ligands can function both as electron donors and electron accepters, and thus will likely increase the oxidation rates of the complexed U(IV) when exposed to oxygen. It is therefore important that the reduced U(IV)-ligand complexes remain away from oxidants including oxygen to prevent them from reoxidizing and thus remobilizing.

2.6 Influence of Siderophores

Microbial siderophores are a group of organic ligands that may have an important effect on tetravalent actinide mobility (Brainard et al., 1992; Ruggiero et al., 2002). It has been shown that organic ligands can increase the solubility and dissolution kinetics of tetravalent actinide minerals such as PuO₂ (Brainard et al., 1992). Siderophores (e.g., desferrioxamine-B (DFO-B), alcaligin, bisucaberin, putrebactin, rhodotorulic acid, enterobactin, vibriobactin, azotochelin, myxochelin, fluvibactin, and serratiochelin) are structurally diverse (~500 known structures) category of biogenic ligands that are common in the soil environment (Boukhalfa and Crumbliss, 2002; Kraemer and Hering, 2004). Siderophores are mainly released by microbes in environments where the bioavailability of Fe is limited due to the low solubility of iron oxides. Interestingly, tetravalent actinides such as U(IV), Pu(IV), and Th(IV) are similar to Fe(III) in several ways that determine their coordination chemistry (e.g., similar charge to ionic radius ratio and first hydrolysis constants) and their 1:1 complexes with DFO-B can have similarly high stability constants –30.6 for Fe(III), 30.8 for Pu(IV), and 26.6 for Th(IV) (Hernlem et al., 1999). The hexavalent redox state of U is thermodynamically stable in the presence of oxygen; however, greater than 60% of the U spread

into the environment by the Chernobyl accident remained in the tetravalent form up to 12–14 years after the accident (Mironov et al., 2002). This demonstrates that solid U(IV)-bearing phases can exist in oxic environments for decades as a result of slow oxidation kinetics. On these time scales, siderophores might have a significant effect on U(IV) mobility in oxic environments in several ways. The first step of ligand promoted dissolution mechanism is the adsorption of the ligand to the mineral surface (Furrer and Stumm, 1986; Zinder et al., 1986). The maximum adsorbed ligand concentration for DFO-B and UO_2 systems at pH 7.5 was $3.3 \mu\text{mol m}^{-2}$, which followed the Langmuir type isotherms (Frazier et al., 2005). It has been observed that DFO-B effectively accelerates UO_2 dissolution and increases UO_2 solubility. There was fast initial dissolution for about 200 hours before reaching a steady state; however, this fast initial dissolution remained unexplained. This siderophore-controlled dissolution was minimum at pH 7–8, whereas it increased at lower as well as higher pH ranges. The dissolution was not substantially affected by the introduction of Fe(III), although theoretically they would compete for the same ligand, indicating no competitive metal-exchange reaction (Frazier et al., 2005). These findings suggest that it is important to consider biogenic organic ligands, including siderophores, when making predictions regarding the stability of U(IV) under reducing conditions.

2.7 Influence of Sizes of Various Forms of U(IV)

Reoxidation of U(IV) will also likely depend on its size as well as its compounds as described by Spycher et al. (2011). It has recently been reported that uraninite is not the only product of direct and indirect biological U(VI) reduction; molecular tetravalent U is also formed. For example, U(IV) produced in *Desulfitobacterium* cultures was not UO_2 , but a phase composed of mononuclear U(IV) atoms closely surrounded by light element shells, where the atomic arrangement likely resulted from inner-sphere bonds between U(IV) and C/N/O- or P/S-containing ligands, such as carbonate or phosphate (Fletcher et al., 2010). The study of U(IV) products formed by enzymatic reduction of *Desulfotomaculum reducens* MI-1, *Clostridium acetobutylicum* (ATCC 824), and *Shewanella oneidensis* MR-1 showed the formation of biomass associated U(IV) complexes, bound most likely through P-containing ligands, as well as other nonuraninite U(IV) species including ningyoite, $\text{U}_2\text{O}(\text{PO}_4)_2$, and $\text{U}_2(\text{PO}_4)(\text{P}_3\text{O}_{10})$ (Bernier-Latmani et al., 2010). The size, nanometer-scale structure, and aggregation state of UO_2 formed by *Shewanella putrefaciens* CN32 were also investigated (Lee et al., 2010). It was shown that the UO_2 nanoparticles formed were highly aggregated with organic polymers. The uraninite nanocrystals were networked in around 100 nm diameter spherical aggregates that

displayed some concentric UO_2 accumulation with heterogeneity. Boyanov et al. (2011) studied the atomic coordination of U(IV) produced by biotic and abiotic pathways in phosphate-free or phosphate amended media. Their extended X-ray absorption fine structure (EXAFS) spectroscopy analyses showed the formation of nonuraninite, phosphate-complexed U(IV) species in phosphate amended media, independent of the biotic or abiotic mode of U(VI) reduction. In the phosphate free medium, U(VI) reduction by *Anaeromyxobacter* or *Shewanella* species resulted in nanoparticulate uraninite, whereas the reduction by *Desulfitobacterium* spp. and by AH_2QDS (a soluble reductant) yielded nonuraninite, carbonate-complexed U(IV) species, suggesting the use of distinct U(VI) reduction mechanisms by these microorganisms.

The formation of nonuraninite U(IV) species has also been observed due to the U(VI) sorption onto Fe(II) minerals followed by reduction (Jang et al., 2008; O'Loughlin et al., 2010; Veeramani et al., 2011). Veeramani et al. (2011) showed the significant influence of the geochemical and mineralogical environment, as well as the interfacial solute-solid chemistry of the reductant solid-phase on the U(VI) reduction products. A greater role of the geochemical environment compared to biological variability on U(IV) products has been suggested (Bernier-Latmani et al., 2010; Fletcher et al., 2010; Lee et al., 2010; Sivaswamy et al., 2011; Veeramani et al., 2011), although the precise reasons for this still remain unclear (Sharp et al., 2011). Uranium phases with distinct atomic structures (and sizes) are likely to have different solubility and mobility that will likely affect the ultimate success of remediation approaches. Therefore, sizes of various molecular U(IV) species must also be considered when modeling U mobility and stability in the subsurface.

3. SUMMARY AND SUGGESTIONS

A number of electron acceptors present in the subsurface e.g., nitrate reduction intermediates (NO_2^- , N_2O , NO), Mn(IV) oxides, and Fe(III)-(hydr)oxides can oxidize biogenic U(IV) to U(VI). For example, oxidation of immobilized U(IV) by Fe(III)-(hydr)oxides has been shown under sulfate-reducing, electron donor-limited conditions. However, detailed molecular mechanisms of uraninite oxidation, especially under reducing conditions, remain unclear. Data suggest that elevated bicarbonate concentrations resulting from OC stimulated microbial respiration favor U(IV) reoxidation/remobilization even under reducing conditions. Siderophores and organic ligands present in the subsurface can also increase the dissolution and solubility of U(IV). The various factors involved in reoxidation of bioreduced uranium in the subsurface are summarized in Figure 2. As discussed previously, biogenic U(IV) can be oxidized by biologically mediated processes, thereby resulting in a reversal

of the remediation process. These U(IV) oxidation processes, if not mechanistically evaluated, will likely diminish the value of *in situ* bioremediation of U as compared to traditional *ex situ* methods. Therefore, evaluating various mechanisms of U reoxidation (e.g., how electrons are transferred from U(IV) (solid) to inorganic solid electron acceptors such as Fe(III) and Mn(IV), how bicarbonate favors U reoxidation, and how electron donors affect U reoxidation) are critical to develop mathematical models that accurately predict the behavior of bioreduced U in natural settings.

As discussed previously, in addition to U(IV) reoxidation issues, nanoparticulate uraninite and mononuclear U(IV) phases are vulnerable within groundwater environments (Spycher et al., 2011; Sani et al., 2008; Sani et al., 2006; Sani et al., 2005; Sani et al., 2004). Field studies also suggest that sulfate-reducing conditions play vital roles in controlling the stability of U(IV). Using SRB (*Desulfovibrio desulfuricans* G20 -a model organism), our group's unpublished results show that a significant mass fraction of bioreduced U (55–65%) exists as a mobile phase, and raises several fundamental questions regarding long-term U reclamation stewardship. We have also shown that that SRB reduce U(VI) to nanometer-sized uraninite particles (1–5 nm), which reside extracellularly and intracellularly (Sani et al., 2006). The accumulation of uraninite inside the cell (periplasm and cytoplasm) may physically shield bioreduced U(IV) from subsequent oxidation processes, thus furthering the sequestering abilities of bioreduced and immobilized U(IV). Extracellular U(IV) has been shown to be oxidized quickly due to a large surface area to mass ratio of the nanoparticles (Spycher et al., 2011). At U-contaminated sites prior to biostimulation, naturally occurring Fe(III)-(hydr)oxide minerals are considered primary controls for U(VI) transport (Fredrickson et al., 2000; Fredrickson et al., 2002). After U bioreduction, in addition to Fe(III)-(hydr)oxide minerals, bacterial cells (planktonic and biofilm) and secondary minerals formed during U bioreduction (e.g., Fe(II), sulfides, reduced organic matter, electron shuttles) could be controls of biogenic U(IV) transport within environmental systems. Thus, for the successful application of U bio-stabilization, it is also vital to understand the governing biogeochemical factors that control the redox behavior of these extracellular bioreduced uraninite species with respect to U fate, transport, and long-term stability. It is necessary to elucidate biogeochemical factors that control bioreduced U fate, transport, and long-term stability through a series of batch, column, and biogeochemical modeling studies (Figure 3). The research should be focused on characterizing U transport potential by identifying nanocolloidal, colloidal, and immobile fractions of bioreduced U under environmentally relevant biochemical conditions. The experimental data obtained should be used to develop a numerical biogeochemical model to predict the stability of bioreduced U (mononuclear U(IV) phases, uraninite) including colloidal transport.

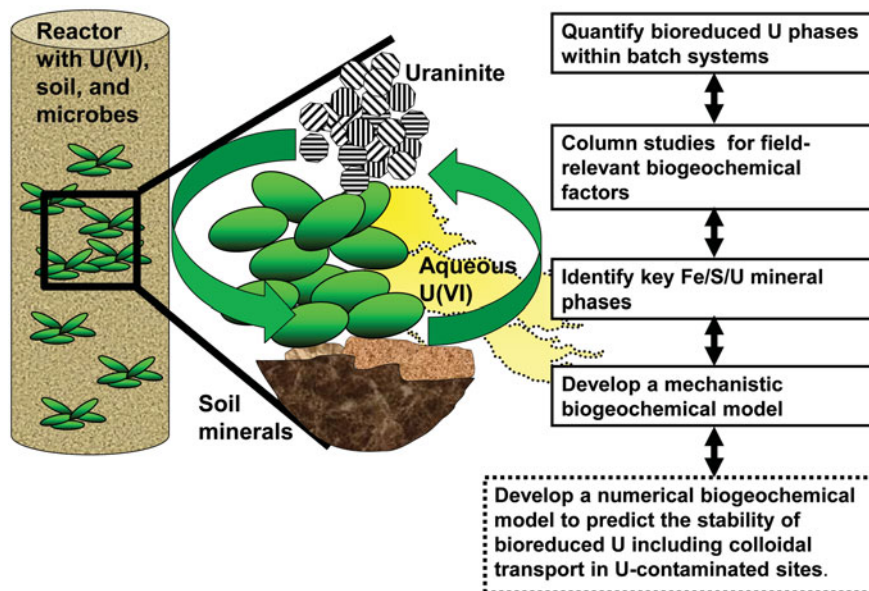


FIGURE 3. Need of a fundamental, mechanistic understanding of the complex interactions among iron minerals, colloids, microbes, and U.

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