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Bioremediation of uranium-contaminated groundwater: a systems approach to subsurface biogeochemistry

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Adding organic electron donors to stimulate microbial reduction of highly soluble U(VI) to less soluble U(IV) is a promising strategy for immobilizing uranium in contaminated subsurface environments. Studies suggest that diagnosing the *in situ* physiological status of the subsurface community during uranium bioremediation with environmental transcriptomic and proteomic techniques can identify factors potentially limiting U(VI) reduction activity. Models which couple genome-scale *in silico* representations of the metabolism of key microbial populations with geochemical and hydrological models may be able to predict the outcome of bioremediation strategies and aid in the development of new approaches. Concerns remain about the long-term stability of sequestered U(IV) minerals and the release of co-contaminants associated with Fe(III) oxides, which might be overcome through targeted delivery of electrons to select microorganisms using *in situ* electrodes.

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Introduction

Uranium contamination of groundwater is extensive worldwide and prohibitively expensive to remediate with traditional strategies, such as pump-and-treat. A promising alternative is to promote microbial reduction of U(VI) to U(IV), which removes uranium from groundwater as an insoluble precipitate (Figure 1) [1••,2••,3]. This constitutes a more attractive option than other biological alternatives, such as biosorption or bioaccumulation [4], due to the greater stability of the reduced form [5]. The effectiveness of microbial reduction for removing uranium from contaminated groundwater has been repeatedly validated across a range of environments, including saturated alluvial sediments [2••] and fractured saprolite [6], with remediation of environments impacted by uranium mining via *in situ* recovery (ISR) gaining considerable interest [7].

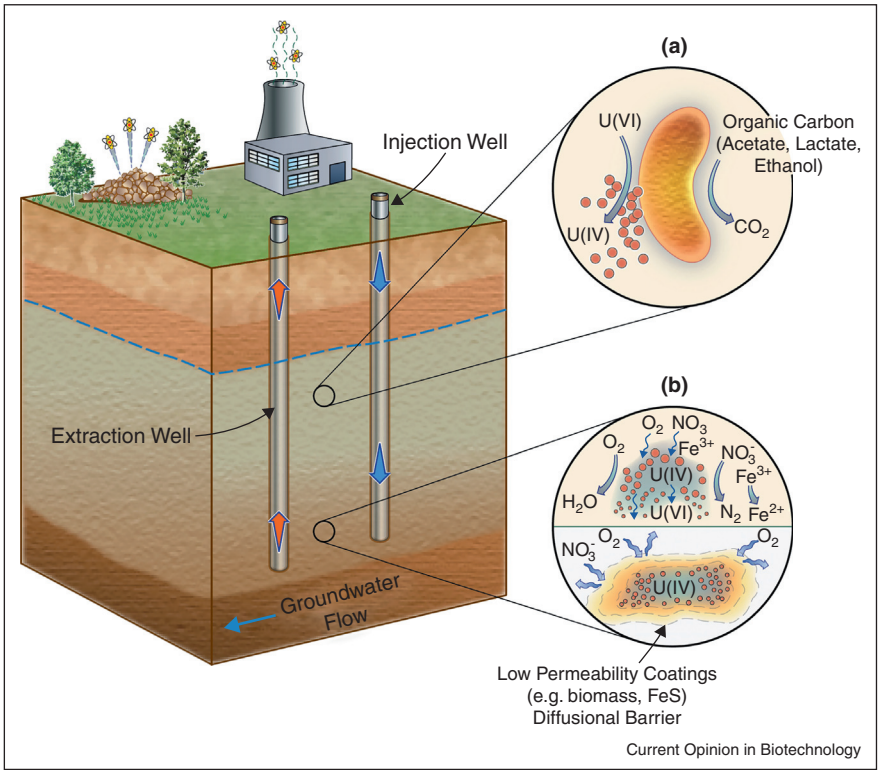
In the twenty years since the discovery of microbial U(VI) reduction, there has been significant progress in understanding the interplay of biology, hydrology, and geochemistry, to the point where in some instances it is possible to predictively model the coupled processes in subsurface environments [8,9]. However, significant questions remain about how to best optimize *in situ* uranium bioremediation because of important knowledge gaps about microbe–microbe and microbe–mineral interactions, as well as a need for more information regarding uranium geochemistry in subsurface environments.

Microorganisms associated with subsurface U(VI) reduction

A broad diversity of microorganisms available in pure culture is capable of U(VI) reduction (Figure 2) [1••,4]. Most are anaerobes that have the ability to reduce other metals, most notably Fe(III), which often represents the most abundant electron acceptor in subsurface environments [9]. It is now recognized that not only vegetative cells, but in some instances spores, can catalyze U(VI) reduction [10•,11]. Recently recognized U(VI)-reducing microorganisms include: *Pseudomonas* sp., *Pantoea* sp. and *Enterobacter* sp. recovered from the soil of a uranium mine [12]; several *Geobacter* species isolated from contaminated sites [9,13]; and the thermophile *Thermus scotoductus* [14•].

T. scotoductus contains a putative peptide ABC transporter, peptide-binding protein capable of U(VI) reduction [14•]. It was concluded that U(VI) reduction by this protein was fortuitous. Promiscuous reduction of U(VI) by proteins with other redox functions is probably a common theme in microbial U(VI) reduction, because, although microbial U(VI) can be driven by natural organic matter in sedimentary environments [15•], levels of U(VI) in uncontaminated waters are typically so low that it is unlikely that specific U(VI) respiratory pathways have evolved [3]. For example, reduction of U(VI) by *Geobacter* species appears to be quite non-specific, with a diversity of outer-surface

Figure 1



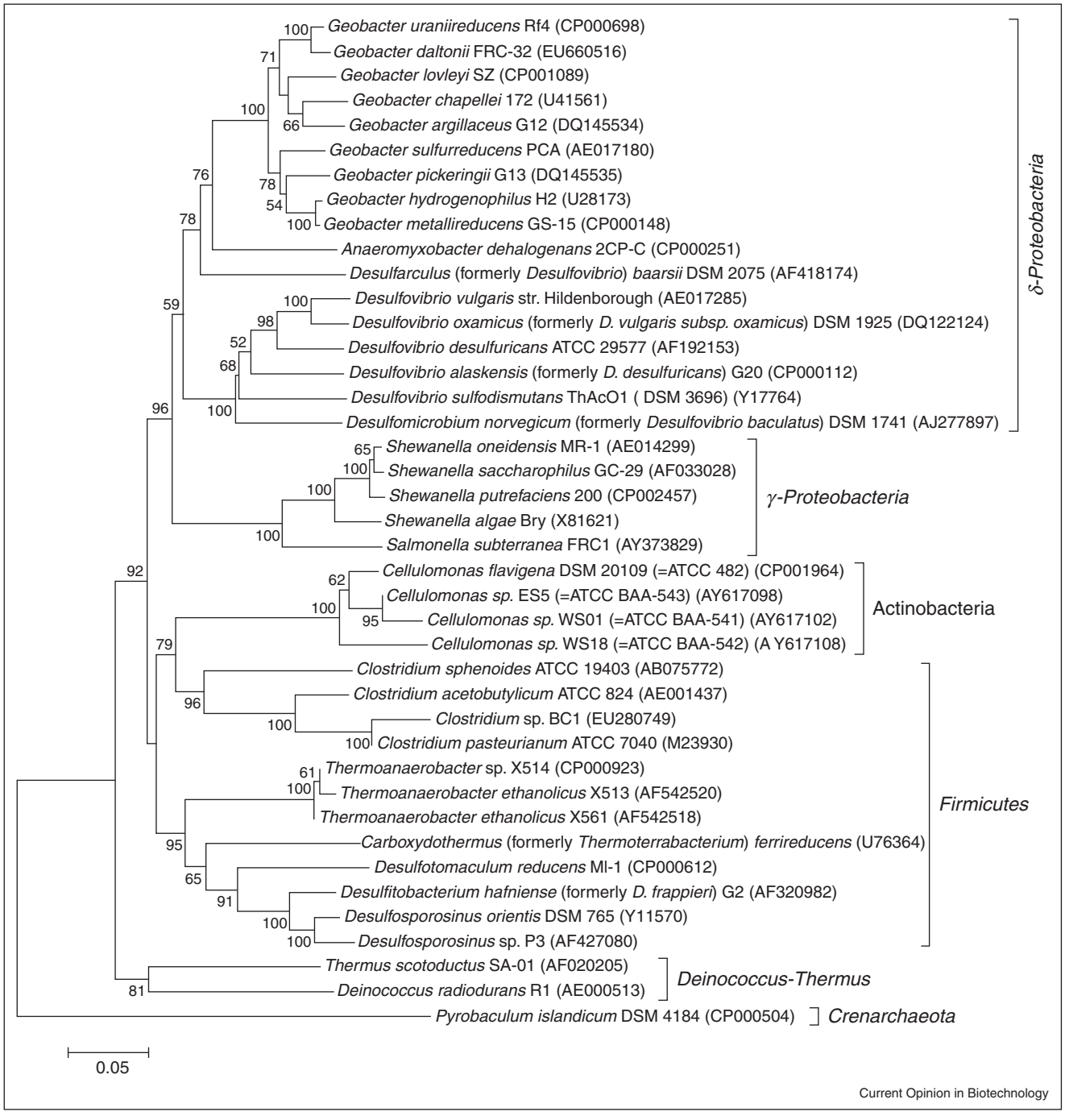
Conceptual illustration of the process of uranium bioremediation. **(a)** Indigenous microorganisms present in soils, sediments, and groundwater contaminated by nuclear energy and weapons production activities are stimulated through introduction of organic carbon compounds via injection wells. Select organisms may couple the oxidation of organic carbon (and H_2) to the reduction of aqueous uranium, as U(VI), converting it from a soluble to an insoluble form, as U(IV). **(b)** Reduced U(IV) may be re-oxidized to U(VI) following cessation of organic carbon injection accompanying subsequent delivery of oxidants, such as O_2, NO_3^-, and Fe^{3+}; the presence of diffusional barriers (e.g., biomass or low permeability sediments) or preferential reductants (e.g., FeS) can suppress re-oxidation and maintain stability of immobilized U(IV).

c-type cytochromes capable of transferring electrons to U(VI) [9] in a manner similar to that reported for humic substances [16]. *Geobacter* species have the ability to reduce electron acceptors at substantial distance from the cell via pili with metallic-like conductivity [17,18] and an associated *c*-type cytochrome [19], but the suggestion that pili are the major site for U(VI) reduction [20] is inconsistent with multiple lines of evidence that suggest that pili are not required for U(VI) reduction [21].

It is difficult to definitively determine which microorganisms are responsible for U(VI) reduction in most subsurface environments, as the availability of alternative electron acceptors capable of supporting anaerobic respiration, such as nitrate, Fe(III), or sulfate [1••,3], is typically much greater than that of U(VI), even in contaminated environments. Thus, although it is common to ascribe the major role in U(VI) reduction to the most dominant organisms in the groundwater during uranium bioremediation, in reality a minor component of the community may be sufficiently abundant to account for the observed U(VI) reduction. Although many studies have focused on planktonic populations and the U(VI) in groundwater, microorganisms affixed to subsurface sediments can play an important role in U(VI) reduction, as U(VI) adsorbed onto sediments can also be reduced, converting it to forms less prone to desorption and release to groundwater [22•,23] (Figure 3).

Many environmental conditions (e.g., type of organic substrates added, pH, salinity, *etc.*) influence which microorganisms predominate during *in situ* uranium bioremediation [24,25]. In some instances, molecular microbial community analysis has revealed rather complex microbial communities associated with bioremediation of uranium-contaminated groundwater [1••]. This was apparent in different treatment zones at the U.S. Department of Energy's (DOE's) Integrated Field Research Challenge (IFRC) site at Oak Ridge, Tennessee (USA). Groundwater at 'Area 3', which had low pH and high nitrate in addition to uranium contamination, was treated with an inner U(VI) bioreduction loop, nested within an outer groundwater-conditioning loop. Geochip analyses revealed distinct microbial communities in the

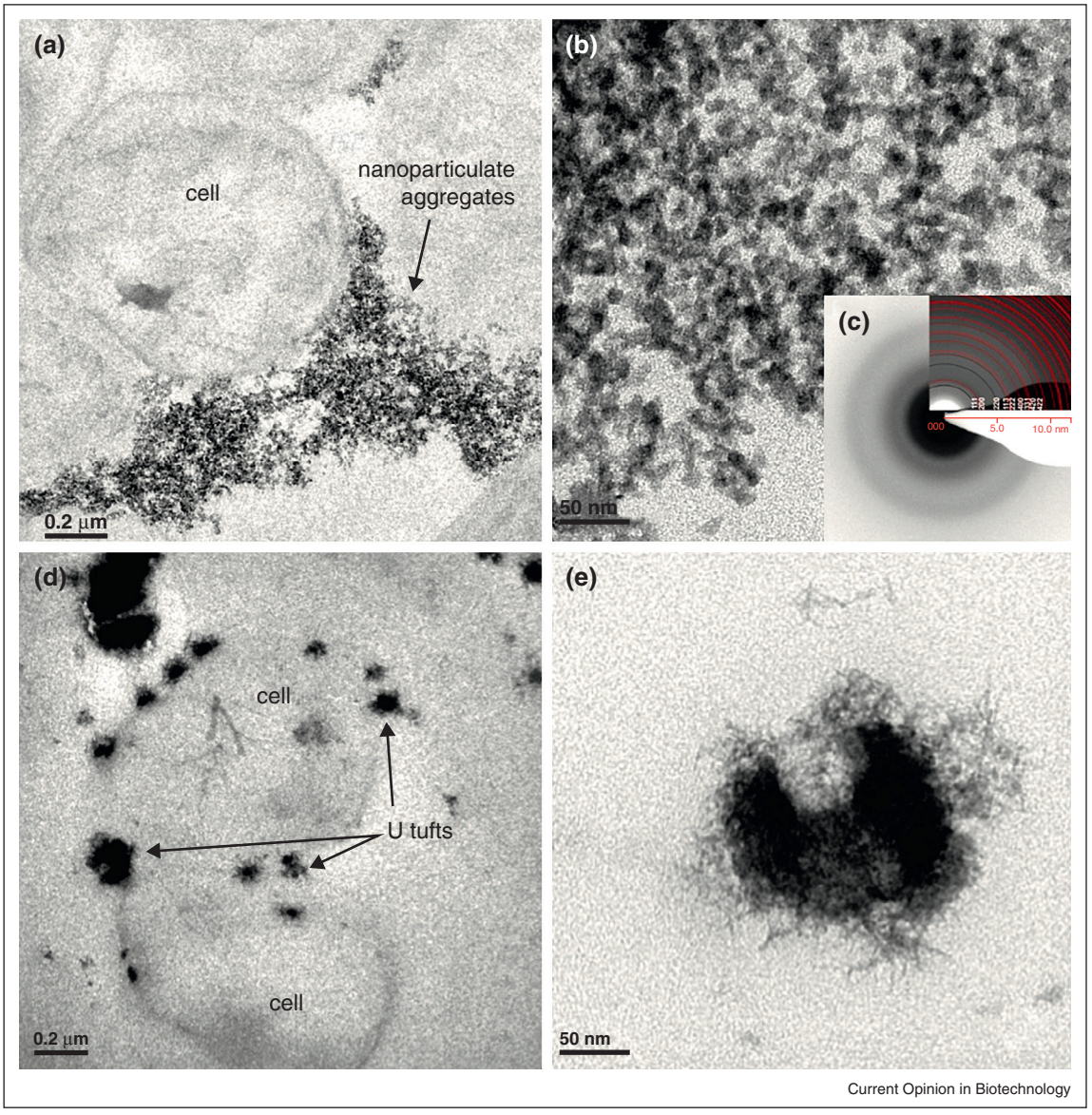
Figure 2



16S rRNA based phylogenetic tree of U(VI)-reducing prokaryotes.
Adapted from Kostka and Green [1••].

two components of the treatment system, with metal-reducing bacteria, such as *Desulfovibrio*, *Geobacter*, *Anaeromyxobacter* and *Shewanella* species highly abundant in the inner *in situ* U(VI)-reduction zone, which received ethanol as an electron donor [26,27]. A massively parallel sequencing-indicator species approach identified *Desulfovibrio*, *Anaeromyxobacter*, and *Desulfosporosinus* species as the predominant organisms [28]. Microorganisms in the families *Burkholderiaceae*, *Comamonadaceae*, *Oxalobacteraceae*, and *Rhodocyclaceae* predominated when microbial activity was stimulated with ethanol and methanol in sediment incubations [24].

Figure 3



Products of U(VI) bioreduction. Comparison of *Shewanella oneidensis* cells **(a–c)** and associated nanoparticulate uraninite (UO_2) to *S. oneidensis* cells exhibiting localized deposits of poorly ordered U(IV) coordination polymers **(d,e)**; scale bars in (d,e) are 200 and 50 nm, respectively. U(IV) morphologies are distinct, with U(IV) coordination polymers staining tuft-like masses on the cells. Images are bright-field transmission electron micrographs; (c) is a selected area electron diffraction pattern interpreted as uraninite.
Adapted with permission from Bernier-Latmani *et al.* [72••].

In studies at 'Area 2', which has circumneutral pH and lower nitrate levels, *Actinobacteria* were the most active under metal-reducing conditions in assimilating ^{13}C from the ^{13}C-ethanol added to promote microbial activity in laboratory sediment incubations [29]. Other laboratory studies with sediments from the Oak Ridge site demonstrated that the type of electron donor added and sediment origin greatly influenced which microorganisms predominated following addition of electron donors [24]. In a field study at this site, addition of emulsified vegetable oil stimulated nitrate, Fe(III), U(VI), and sulfate reduction resulting in a succession of members of the *Comamonadaceae*, *Geobacteraceae*, and *Desulfobacterales* [30].

In contrast to the complexity of the microbial community at the Oak Ridge site, a much simpler microbial community has been noted during the most active phase of U(VI) reduction at the DOE's IFRC site at Rifle, CO, USA [2••]. This may in part be attributed to the circumneutral pH, negligible nitrate, and the fact that acetate, a

non-fermentable substrate, served as the electron donor to promote U(VI) reduction. Increasingly sophisticated molecular analyses performed using a field-portable microarray analysis system [31], Geochip [32] and PhyloChip technologies [33], environmental proteomics [34–36], and analysis of ^{13}C-acetate incorporation into lipids [37] and DNA [2••,22•] have confirmed the findings derived from earlier 16S rRNA gene sequence analyses that *Geobacter* species can account for up to 90% of the 16S rRNA sequences recovered from groundwater during active U(VI) reduction [3,9]. *Geobacter* species possess a number of physiological characteristics that may favor their growth in anaerobic subsurface environments [1••,9,38], including the recently recognized capability to grow as electron-donating partners in syntrophic relationships when Fe(III) is not available [39,40]. At the Rifle site, *Geobacter* species initially grow with Fe(III) as their primary electron acceptor; however, their ability to effectively reduce U(VI) [9] for sustained periods of time suggests that they may also be the primary U(VI) reducers when iron and sulfate reduction (and perhaps methanogenesis) are the predominant metabolic pathways, supplementing U(VI) reduction with reduction of elemental sulfur or syntrophic growth [2••].

Systems analysis and genome-scale modeling of uranium bioremediation

The relative simplicity of the microbial community associated with uranium bioremediation at the Rifle site has made it feasible to analyze and model the *in situ* activity of important subsurface populations and develop tools that can now be potentially applied to more complex bioremediation sites. As recently reviewed [9], a series of studies quantifying transcript abundance of key genes in subsurface *Geobacter* populations elucidated important physiological parameters, such as rates of metabolism [2••], growth rates [9], limitations for key nutrients, such as electron donor [41], phosphate [42], and fixed nitrogen [43], as well as other environmental stresses [43]. Environmental proteomic studies confirmed the abundance of *Geobacter* associated with peak rates of U(VI) reduction and provided information on relative metabolic activity [34,35,44]. The ability of *Geobacter* species to utilize electrodes as electron acceptors has made it feasible to also monitor their *in situ* rates of metabolism by emplacing electrodes in the subsurface [45].

Ideally, bioremediation strategies will be designed in the future with models that can accurately predict the outcome of bioremediation before field implementation, enabling optimization of bioremediation approaches. For example, it may be desirable to titrate in the minimum amount of electron donor necessary to promote effective U(VI) reduction, without producing excess biomass that may reduce permeability [2••] or stimulate the growth of organisms, such as acetate-oxidizing sulfate reducers [46] that consume added electron donor but do not contribute to U(VI) reduction. Models for describing U(VI) reduction in subsurface sediments are being developed with several different approaches [47••,48–51].

Development of truly predictive models will require the ability to forecast how key components of the microbial community respond to changing environmental conditions during bioremediation. One potential approach is Bottom-Up Genome Scale (BUGS) modeling in which genome-scale metabolic models of key organisms influencing the bioremediation process are coupled to geochemical and hydrological models [9]. An advantage of BUGS modeling is that it accounts for important changes in microbial physiology (e.g., growth yields) associated with the changing environmental conditions that microorganisms experience during uranium bioremediation; such features are not represented in traditional approaches for modeling microbial activity. BUGS modeling has been effective in predicting the course of uranium bioremediation in the relatively simple case of acetate-driven uranium bioremediation by coupling genome-scale models of *Geobacter* with reactive transport models [8,52–55,56•,57].

BUGS modeling can describe more complex microbial communities with the successive addition of genome-scale models representing additional key populations. For example, it greatly benefits *in situ* uranium bioremediation that *Geobacter* species, which are capable of U(VI) reduction, outcompete acetate-oxidizing Fe(III)-reducing *Rhodoferax* species (reportedly incapable of U(VI) reduction) when acetate is added to the subsurface. Following development of a *Rhodoferax* genome-scale model [58], BUGS modeling predicted the ability of *Rhodoferax* to effectively compete with *Geobacter* in the subsurface before acetate amendments and elucidated the physiological features that allow *Geobacter* to outcompete *Rhodoferax* when high concentrations of acetate are made available [56•]. Modeling the interaction between *Geobacter* species and acetate-oxidizing sulfate reducers that compete for acetate, but do not reduce U(VI), improved the understanding of observed patterns of U(VI) reduction during acetate amendment [57] and suggested a strategy for enhancing uranium bioremediation through Fe(III) addition designed to maintain the long-term U(VI)-reducing activity of *Geobacter* species [59]. The ability of genome-scale metabolic modeling to predict mutations that change metabolic fluxes to adapt to new substrates may represent an additional predictive power for optimizing bioremediation strategies [60].

The BUGS modeling approach requires a substantial initial investment in isolating and characterizing microorganisms in order to obtain the physiological data required for modeling. In the future, this may be expedited by major advances in metagenomics that, as recently illustrated at the Rifle site, are providing

important basic information on uncultured organisms that should facilitate identification of the appropriate strategies for recovering these organisms in culture [61••]. Ultimately, BUGS modeling can make the design of bioremediation strategies far more predictive and scientific, removing the substantial empiricism associated with the typical current practices for bioremediation of uranium and other contaminants.

Geochemical factors influencing uranium bioremediation

Also crucial for predicting the success of microbial U(VI) reduction as a remediation strategy is an understanding of the abiotic factors influencing the stability of reduced uranium species. At circumneutral pH, U(VI) in groundwater may primarily be complexed with inorganic carbon [2••] in forms that are less susceptible to microbial U(VI) reduction than U(VI) in organic complexes [62,63], which are more readily reduced via enzymatic processes than with potential abiotic reductants. Although reduced iron [64,65] and sulfur [66,67] species can reduce U(VI) under laboratory conditions where inorganic constituents, such as Ca^{2+} and HCO_3^-, are included, incubation at environmentally relevant concentrations severally inhibits (or suppresses) reduction [68•]. This is consistent with field observations that U(VI) is only effectively removed when enzymatic processes are likely to be operative [2••].

Although the most studied product of microbial U(VI) reduction is nanoparticulate (diameter <3-nm) uraninite [69–71], recent studies have reported poorly ordered coordination polymers of U(IV) coordinated to phosphoryl and/or carboxylate groups on biomass [72••] or mineral phases, such as ningyoite, $CaU(PO_4)_2$ [71,72••,73••,74]. The chemical and physical forms of U(IV) and the geochemical environment influence the susceptibility of U(IV) to oxidation and mobilization via the formation of complexes [75–80]. While susceptibility of biogenic uraninite to oxidation is diminished by structural incorporation of Mn^{2+} [81] and reaction with Ca^{2+} [82], the most important stabilizing mechanisms (see Figure 1B) appear to be maintenance of low levels of dissolved oxygen and/or sequestration within low permeability sediments or enveloping biomass [76].

Concerns about potential re-mobilization of uranium precipitated as U(IV) illustrate the important limitation of microbial U(VI) reduction as a bioremediation strategy, which is that uranium remains in the subsurface. Other potential drawbacks are loss of aquifer permeability due to biomass and mineral accumulation [2••], which can impede prolonged delivery of organic carbon, and the release of toxic contaminants, such as arsenic [83], adsorbed onto Fe(III) oxides.

An alternative that overcomes these limitations is to promote microbial U(VI) reduction by emplacing electrodes that serve as the electron donor for the reduction of U(VI) [3,84]. Negatively poised electrodes provide a constant, steady source of electrons without promoting significant microbial Fe(III) or sulfate reduction and the deleterious water quality issues associated with those reactions. Furthermore, the U(IV) produced in electrode-driven microbial U(VI) reduction precipitates on the electrodes, facilitating removal of uranium from the subsurface. Although not predicated on reduction, alternative mechanisms for uranium bio-immobilization, such as biomineralization of insoluble U(VI)-phosphate species [85•] through microbial phosphatase stimulation, continue to attract interest for subsurface remediation.

Conclusions

Promoting microbial U(VI) reduction has proven to be an effective strategy for removing uranium from contaminated groundwater in the short term, thereby preventing its mobility. However, there is still significant uncertainty about the long-term sustainability of this approach and many options that might optimize the process have yet to be evaluated. An important outcome of the intensive investment in uranium bioremediation research in the last decade has been the development of approaches for assessing and modeling the activity of subsurface microbial communities that are expected to be applicable to the other forms of subsurface bioremediation, as well as to the study of diverse microbial processes in a wide range of soils and sediments.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. •• Kostka JE, Green SJ: **Microorganisms and processes linked to uranium reduction and immobilization**. In *Microbial Metal and Metalloid Metabolism: Advances and Applications.* Edited by Stolz JF, Oremland RS. ASM Press; 2011:117-138.
Excellent overview of the topic that also includes important older references.

2. •• Williams KH, Long PE, Davis JA, Wilkins MJ, N'Guessan AL, Steefel CI, Yang L, Newcomer D, Spane FA, Kerkhof LJ *et al.*: **Acetate availability and its influence on sustainable bioremediation of uranium-contaminated groundwater**. *Geomicrobiol J* 2011, **28**:519-539.

A comprehensive overview of recent stimulated uranium bioreduction field campaigns at the U.S. Department of Energy's Integrated Field Research Challenge (IFRC) site at Rifle, CO, USA.

3. Lovley DR: **Live wires: direct extracellular electron exchange for bioenergy and the bioremediation of energy-related contamination**. *Energ Environ Sci* 2011, **4**:4896.

4. Mohapatra BR, Dinardo O, Gould WD, Koren DW: **Biochemical and genomic facets on the dissimilatory reduction of radionuclides by microorganisms — a review**. *Miner Eng* 2010, **23**:591-599.

5. Cuney M: **Evolution of uranium fractionation processes through time: driving the secular variation of uranium deposit types**. *Econ Geol* 2010, **105**:553-569.

6. Wu W-M, Carley J, Green SJ, Luo J, Kelly SD, Nostrand JV, Lowe K, Mehlhorn T, Carroll S, Boonchayanant B *et al.*: **Effects of nitrate on the stability of uranium in a bioreduced region of the subsurface**. *Environ Sci Technol* 2010, **44**:5104-5111.

7. Borch T, Roche N, Johnson TE: **Determination of contaminant levels and remediation efficacy in groundwater at a former in situ recovery uranium mine**. *J Environ Monit* 2012, **14**:1814-1823.

8. Mahadevan R, Palsson BO, Lovley DR: **In situ to in silico and back: elucidating the physiology and ecology of *Geobacter* spp. Using genome-scale modelling**. *Nat Rev Microbiol* 2011, **9**:39-50.

9. Lovley DR, Ueki T, Zhang T, Malvankar NS, Shrestha PM, Flanagan KA, Aklujkar M, Butler JE, Giloteaux L, Rotaru AE *et al.*: ***Geobacter*: the microbe electric's physiology, ecology, and practical applications**. *Adv Microb Physiol* 2011, **59**:1-100.

10. • Junier P, Frutschi M, Wigginton NS, Schofield EJ, Bargar JR, Bernier-Latmani R: **Metal reduction by spores of *Desulfotomaculum reducens***. *Environ Microbiol* 2009, **11**:3007-3017.
This paper demonstrates that metabolically inactive spores may also contribute to process of uranium reduction.

11. Vecchia ED, Veeramani H, Suvorova EI, Wigginton NS, Bargar JR, Bernier-Latmani R: **U(VI) reduction by spores of *Clostridium acetobutylicum***. *Res Microbiol* 2010, **161**:765-771.

12. Chabalala S, Chirwa EMN: **Removal of uranium(VI) under aerobic and anaerobic conditions using an indigenous mine consortium**. *Miner Eng* 2010, **23**:526-531.

13. Prakash O, Gihring TM, Dalton DD, Chin KJ, Green SJ, Akob DM, Wanger G, Kostka JE: ***Geobacter daltonii* sp. nov., an Fe(III)- and uranium(VI)-reducing bacterium isolated from a shallow subsurface exposed to mixed heavy metal and hydrocarbon contamination**. *Int J Syst Evol Microbiol* 2010, **60**:546-553.

14. • Cason ED, Piater LA, van Heerden E: **Reduction of U(VI) by the deep subsurface bacterium, *Thermus scotoductus* SA-01, and the involvement of the ABC transporter protein**. *Chemosphere* 2012, **86**:572-577.
This study used classical molecular biology techniques to identify a potentially new role for a peptide ABC transporter protein in uranium reduction in a *Thermus* species, in contrast to cytochrome-mediated mechanisms in other well studied U(VI)-reducing bacteria.

15. • Campbell KM, Kukkadapu RK, Qafoku NP, Peacock AD, Lesher E, Williams KH, Bargar JR, Wilkins MJ, Figueroa L, Ranville J *et al.*: **Geochemical, mineralogical and microbiological characteristics of sediment from a naturally reduced zone in a uranium-contaminated aquifer**. *Appl Geochem* 2012, **27**:1499-1511.
This study presents a natural analogue for engineered uranium bioremediation, suggesting that the process may occur where low molecular weight organic acids are produced naturally and validating a model for redox-mediated uranium ore genesis.

16. Voordeckers JW, Kim BC, Izallalen M, Lovley DR: **Role of *Geobacter sulfurreducens* outer surface c-type cytochromes in reduction of soil humic acid and anthraquinone-2,6-disulfonate**. *Appl Environ Microbiol* 2010, **76**:2371-2375.

17. Malvankar NS, Vargas M, Nevin KP, Franks AE, Leang C, Kim BC, Inoue K, Mester T, Covalla SF, Johnson JP *et al.*: **Tunable metallic-like conductivity in microbial nanowire networks**. *Nat Nanotechnol* 2011, **6**:573-579.

18. Malvankar NS, Lovley DR: **Microbial nanowires: a new paradigm for biological electron transfer and bioelectronics**. *Chemsuschem* 2012, **5**:1039-1046.

19. Leang C, Qian XL, Mester T, Lovley DR: **Alignment of the c-type cytochrome OmcS along pili of *Geobacter sulfurreducens***. *Appl Environ Microbiol* 2010, **76**:4080-4084.

20. Cologgi DL, Lampa-Pastirk S, Speers AM, Kelly SD, Reguera G: **Extracellular reduction of uranium via *Geobacter* conductive pili as a protective cellular mechanism**. *Proc Natl Acad Sci U S A* 2011, **108**:15248-15252.

21. Orellana R, Leavitt J, Comolli L, Leang C, Gray A, Lovley DR: **Outer surface cytochromes, not pili, are the primary conduit for U(VI) reduction in *Geobacter sulfurreducens***. *Abstracts of the 2012 Annual Meeting of the Society for Microbiology*. 2012.

22. • Kerkhof LJ, Williams KH, Long PE, McGuinness LR: **Phase preference by active, acetate-utilizing bacteria at the Rifle, CO integrated field research challenge site**. *Environ Sci Technol* 2011, **45**:1250-1256.
Demonstrates the importance of microbial populations attached to sediment particles in uranium bioremediation.

23. Begg JDC, Burke IT, Lloyd JR, Boothman C, Shaw S, Charnock JM, Morris K: **Bioreduction behavior of U(VI) sorbed to sediments**. *Geomicrobiol J* 2011, **28**:160-171.

24. Vishnivetskaya TA, Brandt CC, Madden AS, Drake MM, Kostka JE, Akob DM, Kusel K, Palumbo AV: **Microbial community changes in response to ethanol or methanol amendments for U(VI) reduction**. *Appl Environ Microbiol* 2010, **76**:5728-5735.

25. Barlett M, Moon HS, Peacock AA, Hedrick DB, Williams KH, Long PE, Lovley D, Jaffe PR: **Uranium reduction and microbial community development in response to stimulation with different electron donors**. *Biodegradation* 2012, **23**:535-546.

26. Xu M, Wu WM, Wu L, He Z, Van Nostrand JD, Deng Y, Luo J, Carley J, Ginder-Vogel M, Gentry TJ *et al.*: **Responses of microbial community functional structures to pilot-scale uranium in situ bioremediation**. *ISME J* 2010, **4**:1060-1070.

27. Van Nostrand JD, Wu L, Wu WM, Huang Z, Gentry TJ, Deng Y, Carley J, Carroll S, He Z, Gu B *et al.*: **Dynamics of microbial community composition and function during in situ bioremediation of a uranium-contaminated aquifer**. *Appl Environ Microbiol* 2011, **77**:3860-3869.

28. Cardenas E, Wu WM, Leigh MB, Carley J, Carroll S, Gentry T, Luo J, Watson D, Gu B, Ginder-Vogel M *et al.*: **Significant association between sulfate-reducing bacteria and uranium-reducing microbial communities as revealed by a combined massively parallel sequencing-indicator species approach**. *Appl Environ Microbiol* 2010, **76**:6778-6786.

29. Akob DM, Kerkhof L, Kusel K, Watson DB, Palumbo AV, Kostka JE: **Linking specific heterotrophic bacterial populations to bioreduction of uranium and nitrate in contaminated subsurface sediments by using stable isotope probing**. *Appl Environ Microbiol* 2011, **77**:8197-8200.

30. Gihring TM, Zhang G, Brandt CC, Brooks SC, Campbell JH, Carroll S, Criddle CS, Green SJ, Jardine P, Kostka JE *et al.*: **A limited microbial consortium is responsible for extended bioreduction of uranium in a contaminated aquifer**. *Appl Environ Microbiol* 2011, **77**:5955-5965.

31. Chandler DP, Kukhtin A, Mokhiber R, Knickerbocker C, Ogles D, Rudy G, Golova J, Long P, Peacock A: **Monitoring microbial community structure and dynamics during in situ U(VI) bioremediation with a field-portable microarray analysis system**. *Environ Sci Technol* 2010, **44**:5516-5522.

32. Liang YT, Van Nostrand JD, N'Guessan LA, Peacock AD, Deng Y, Long PE, Resch CT, Wu LY, He ZL, Li GH *et al.*: **Microbial functional gene diversity with a shift of subsurface redox conditions during in situ uranium reduction**. *Appl Environ Microbiol* 2012, **78**:2966-2972.

33. Handley KM, Wrighton KC, Piceno YM, Andersen GL, DeSantis TZ, Williams KH, Wilkins MJ, N'Guessan AL, Peacock A, Bargar J *et al.*: **High-density phylochip profiling of stimulated aquifer**

microbial communities reveals a complex response to acetate amendment. *FEMS Microbiol Ecol* 2012, **81**:188-204.

34. Wilkins MJ, Verberkmoes NC, Williams KH, Callister SJ, Mouser PJ, Elifantz H, N'Guessan AL, Thomas BC, Nicora CD, Shah MB *et al.*: **Proteogenomic monitoring of *Geobacter* physiology during stimulated uranium bioremediation**. *Appl Environ Microbiol* 2009, **75**:6591-6599.

35. Wilkins MJ, Callister SJ, Miletto M, Williams KH, Nicora CD, Lovley DR, Long PE, Lipton MS: **Development of a biomarker for *Geobacter* activity and strain composition; proteogenomic analysis of the citrate synthase protein during bioremediation of U(VI)**. *Microb Biotechnol* 2011, **4**:55-63.

36. Callister SJ, Wilkins MJ, Nicora CD, Williams KH, Banfield JF, VerBerkmoes NC, Hettich RL, N'Guessan L, Mouser PJ, Elifantz H *et al.*: **Analysis of biostimulated microbial communities from two field experiments reveals temporal and spatial differences in proteome profiles**. *Environ Sci Technol* 2010, **44**:8897-8903.

37. Peacock AD, Hedrick DB, Long PE, Nevin KP, Resch CT, Lovley DR, White DC: **Field-scale uranium (VI) bioimmobilization monitored by lipid biomarkers and ^{13}C-acetate incorporation**. *Remed J* 2011, **21**:85-106.

38. Helmus RA, Liermann LJ, Brantley SL, Tien M: **Growth advantage in stationary-phase (GASP) phenotype in long-term survival strains of *Geobacter sulfurreducens***. *FEMS Microbiol Ecol* 2012, **79**:218-228.

39. Morita M, Malvankar NS, Franks AE, Summers ZM, Giloteaux L, Rotaru AE, Rotaru C, Lovley DR: **Potential for direct interspecies electron transfer in methanogenic wastewater digester aggregates**. *Mbio* 2011, **2** e00159-00111.

40. Summers ZM, Fogarty HE, Leang C, Franks AE, Malvankar NS, Lovley DR: **Direct exchange of electrons within aggregates of an evolved syntrophic coculture of anaerobic bacteria**. *Science* 2010, **330**:1413-1415.

41. Elifantz H, N'Guessan LA, Mouser PJ, Williams KH, Wilkins MJ, Risso C, Holmes DE, Long PE, Lovley DR: **Expression of acetate permease-like (apl) genes in subsurface communities of *Geobacter* species under fluctuating acetate concentrations**. *FEMS Microbiol Ecol* 2010, **73**:441-449.

42. N'Guessan AL, Elifantz H, Nevin KP, Mouser PJ, Methe B, Woodard TL, Manley K, Williams KH, Wilkins MJ, Larsen JT *et al.*: **Molecular analysis of phosphate limitation in *Geobacteraceae* during the bioremediation of a uranium-contaminated aquifer**. *ISME J* 2010, **4**:253-266.

43. Mouser PJ, N'Guessan AL, Elifantz H, Holmes DE, Williams KH, Wilkins MJ, Long PE, Lovley DR: **Influence of heterogeneous ammonium availability on bacterial community structure and the expression of nitrogen fixation and ammonium transporter genes during in situ bioremediation of uranium-contaminated groundwater**. *Environ Sci Technol* 2009, **43**:4386-4392.

44. Yun J, Ueki T, Miletto M, Lovley DR: **Monitoring the metabolic status of *Geobacter* species in contaminated groundwater by quantifying key metabolic proteins with *Geobacter*-specific antibodies**. *Appl Environ Microbiol* 2011, **77**:4597-4602.

45. Williams KH, Nevin KP, Franks A, Englert A, Long PE, Lovley DR: **Electrode-based approach for monitoring in situ microbial activity during subsurface bioremediation**. *Environ Sci Technol* 2010, **44**:47-54.

46. Miletto M, Williams KH, N'Guessan AL, Lovley DR: **Molecular analysis of the metabolic rates of discrete subsurface populations of sulfate reducers**. *Appl Environ Microbiol* 2011, **77**:6502-6509.

47. •• Jin Q, Roden EE: **Microbial physiology-based model of ethanol metabolism in subsurface sediments**. *J Contam Hydrol* 2011, **125**:1-12.
Excellent example of modeling of complex microbial interactions as applied to ethanol-driven bioremediation.

48. Fang Y, Yabusaki SB, Morrison SJ, Amonette JP, Long PE: **Multicomponent reactive transport modeling of uranium bioremediation field experiments**. *Geochim Cosmochim Acta* 2009, **73**:6029-6051.

49. Istok JD, Park M, Michalsen M, Spain AM, Krumholz LR, Liu C, McKinley J, Long P, Roden E, Peacock AD *et al.*: **A thermodynamically-based model for predicting microbial growth and community composition coupled to system geochemistry: application to uranium bioreduction**. *J Contam Hydrol* 2010, **112**:1-14.

50. Yabusaki SB, Fang Y, Williams KH, Murray CJ, Ward AL, Dayvault RD, Waichler SR, Newcomer DR, Spane FA, Long PE: **Variably saturated flow and multicomponent biogeochemical reactive transport modeling of a uranium bioremediation field experiment**. *J Contam Hydrol* 2011, **126**:271-290.

51. Zhang F, Wu WM, Parker JC, Mehlhorn T, Kelly SD, Kemner KM, Zhang G, Schadt C, Brooks SC, Criddle CS *et al.*: **Kinetic analysis and modeling of oleate and ethanol stimulated uranium (VI) bio-reduction in contaminated sediments under sulfate reduction conditions**. *J Hazard Mater* 2010, **183**:482-489.

52. Scheibe TD, Mahadevan R, Fang YL, Garg S, Long PE, Lovley DR: **Coupling a genome-scale metabolic model with a reactive transport model to describe in situ uranium bioremediation**. *Microb Biotechnol* 2009, **2**:274-286.

53. Zhao J, Fang Y, Scheibe TD, Lovley DR, Mahadevan R: **Modeling and sensitivity analysis of electron capacitance for *Geobacter* in sedimentary environments**. *J Contam Hydrol* 2010, **112**:30-44.

54. Fang Y, Scheibe TD, Mahadevan R, Garg S, Long PE, Lovley DR: **Direct coupling of a genome-scale microbial in silico model and a groundwater reactive transport model**. *J Contam Hydrol* 2011, **122**:96-103.

55. Zhao J, Scheibe TD, Mahadevan R: **Model-based analysis of the role of biological, hydrological and geochemical factors affecting uranium bioremediation**. *Biotechnol Bioeng* 2011, **108**:1537-1548.

56. • Zhuang K, Izallalen M, Mouser P, Richter H, Risso C, Mahadevan R, Lovley DR: **Genome-scale dynamic modeling of the competition between *Rhodoferax* and *Geobacter* in anoxic subsurface environments**. *ISME J* 2011, **5**:305-316.
Demonstration of how genome-scale dynamic modeling approaches can be used to evaluate microbial competition and niche exploitation during in situ U(VI) bioremediation.

57. Barlett M, Zhuang K, Mahadevan R, Lovley D: **Integrative analysis of *Geobacter* spp. and sulfate-reducing bacteria during uranium bioremediation**. *Biogeosciences* 2012, **9**:1033-1040.

58. Risso C, Sun J, Zhuang K, Mahadevan R, Deboy R, Ismail W, Shrivastava S, Huot H, Kothari S, Daugherty S *et al.*: **Genome-scale comparison and constraint-based metabolic reconstruction of the facultative anaerobic Fe(III)-reducer *Rhodoferax ferrireducens***. *BMC Genom* 2009:10.

59. Zhuang K, Ma E, Lovley DR, Mahadevan R: **The design of long-term effective uranium bioremediation strategy using a community metabolic model**. *Biotechnol Bioeng* 2012 http://dx.doi.org/10.1002/bit.24528.

60. Summers ZM, Ueki T, Ismail W, Haveman SA, Lovley DR: **Laboratory evolution of *Geobacter sulfurreducens* for enhanced growth on lactate via a single-base-pair substitution in a transcriptional regulator**. *ISME J* 2012, **6**:975-983.

61. •• Wrighton KC, Thomas BC, Sharon I, Miller CS, Castelle CJ, VerBerkmoes NC, Wilkins MJ, Hettich RL, Lipton MS, Williams KH, et al.: **Fermentation, hydrogen and sulfur metabolism in multiple uncultivated bacterial phyla**. *Science*, in press
A detailed analysis of metagenomic data to reveal physiological capabilities of novel, uncultured organisms.

62. Ulrich KU, Veeramani H, Bernier-Latmani R, Giammar DE: **Speciation-dependent kinetics of uranium(VI) bioreduction**. *Geomicrobiol J* 2011, **28**:396-409.

63. Suzuki Y, Tanaka K, Kozai N, Ohnuki T: **Effects of citrate, NTA, and EDTA on the reduction of U(VI) by *Shewanella putrefaciens***. *Geomicrobiol J* 2010, **27**:245-250.

64. Latta DE, Boyanov MI, Kemner KM, O'Loughlin EJ, Scherer MM: **Abiotic reduction of uranium by Fe(II) in soil**. *Appl Geochem* 2012, **27**:1512-1524.

65. Latta DE, Gorski CA, Boyanov MI, O'Loughlin EJ, Kemner KM, Scherer MM: **Influence of magnetite stoichiometry on U-VI reduction**. *Environ Sci Technol* 2012, **46**:778-786.

66. Boonchayaanant B, Gu BH, Wang W, Ortiz ME, Criddle CS: **Can microbially-generated hydrogen sulfide account for the rates of U(VI) reduction by a sulfate-reducing bacterium?** *Biodegradation* 2010, **21**:81-95.

67. Hyun SP, Davis JA, Sun K, Hayes KF: **Uranium(VI) reduction by iron(II) monosulfide mackinawite**. *Environ Sci Technol* 2012, **46**:3369-3376.

68. • Singer DM, Chatman SM, Ilton ES, Rosso KM, Banfield JF, Waychunas GA: **U(VI) sorption and reduction kinetics on the magnetite (1 1 1) surface**. *Environ Sci Technol* 2012, **46**:3821-3830.
An elegant study illustrating the importance of environmental conditions in controlling or suppressing abiotic reductive pathways for uranium; provides compelling support for enzymatic pathways being dominant process responsible for many field uranium bioremediation experiments.

69. Kelly SD, Wu W-M, Yang F, Criddle CS, Marsh TL, O'Loughlin EJ, Ravel B, Watson D, Jardine PM, Kemner KM: **Uranium transformations in static microcosms**. *Environ Sci Technol* 2009, **44**:236-242.

70. Sharp JO, Schofield EJ, Veeramani H, Suvorova EI, Kennedy DW, Marshall MJ, Mehta M, Bargar JR, Bernier-Latmani R: **Structural similarities between biogenic uraninites produced by phylogenetically and metabolically diverse bacteria**. *Environ Sci Technol* 2009, **43**:8295-8301.

71. Lee SY, Baik MH, Choi JW: **Biogenic formation and growth of uraninite (UO_2)**. *Environ Sci Technol* 2010, **44**:8409-8414.

72. •• Bernier-Latmani R, Veeramani H, Vecchia ED, Junier P, Lezama-Pacheco JS, Suvorova EI, Sharp JO, Wigginton NS, Bargar JR: **Non-uraninite products of microbial U(VI) reduction**. *Environ Sci Technol* 2010, **44**:9456-9462.
Documents the importance of solution composition on the form of the products of microbial U(VI) reduction and the ability of both Gram-positive and Gram-negative strains to yield poorly ordered U(IV) coordination polymers.

73. •• Fletcher KE, Boyanov MI, Thomas SH, Wu QZ, Kemner KM, Loffler FE: **U(VI) reduction to mononuclear U(IV) by *Desulfitobacterium* species**. *Environ Sci Technol* 2010, **44**:4705-4709.
Demonstrates that U(VI) reducing bacteria are capable of producing U(IV) coordination polymers, highlighting the potential importance of this U(IV) species in environmental systems and models describing the end products of bioreduction.

74. Boyanov MI, Fletcher KE, Kwon MJ, Rui X, O'Loughlin EJ, Loffler FE, Kemner KM: **Solution and microbial controls on the formation of reduced U(IV) species**. *Environ Sci Technol* 2011, **45**:8336-8344.

75. Alessi DA, Uster B, Veeramani H, Suvorova E, Lezama-Pacheco JS, Stubbs JE, Bargar JR, Bernier-Latmani R: **Quantitative separation of monomeric U(IV) from UO_2 in products of U(VI) reduction**. *Environ Sci Technol* 2012, **46**:6150-6157.

76. Campbell KM, Veeramani H, Urich KU, Blue LY, Giammar DE, Bernier-Latmani R, Stubbs JE, Suvorova E, Yabusaki S, Lezama-Pacheco JS *et al.*: **Oxidative dissolution of biogenic uraninite in groundwater at Old Rifle, CO**. *Environ Sci Technol* 2011, **45**:8748-8754.

77. Ginder-Vogel M, Stewart B, Fendorf S: **Kinetic and mechanistic constraints on the oxidation of biogenic uraninite by ferrihydrite**. *Environ Sci Technol* 2010, **44**:163-169.

78. Moon HS, Komlos J, Jaffe PR: **Biogenic U(IV) oxidation by dissolved oxygen and nitrate in sediment after prolonged U(VI)/Fe(III)/SO_4^{2-} reduction**. *J Contam Hydrol* 2009, **105**:18-27.

79. Ulrich K-U, Ilton ES, Veeramani H, Sharp JO, Bernier-Latmani R, Schofield EJ, Bargar JR, Giammar DE: **Comparative dissolution kinetics of biogenic and chemogenic uraninite under oxidizing conditions in the presence of carbonate**. *Geochim Cosmochim Acta* 2009, **73**:6065-6083.

80. Zhang GX, Burgos WD, Senko JM, Bishop ME, Dong HL, Boyanov MI, Kemner KM: **Microbial reduction of chlorite and uranium followed by air oxidation**. *Chem Geol* 2011, **283**:242-250.

81. Veeramani H, Schofield EJ, Sharp JO, Suvorova EI, Ulrich KU, Mehta A, Giammar DE, Bargar JR, Bernier-Latmani R: **Effect of Mn(II) on the structure and reactivity of biogenic uraninite**. *Environ Sci Technol* 2009, **43**:6541-6547.

82. Cerrato JM, Barrows CJ, Blue LY, Lezama-Pacheco JS, Bargar JR, Giammar DE: **Effect of Ca^{2+} and Zn^{2+} on UO_2 dissolution rates**. *Environ Sci Technol* 2012, **46**:2731-2737.

83. Giloteaux L, Holmes DE, Williams KH, Wrighton KC, Wilkins MJ, Montgomery AP, Smith JA, Orellana R, Thompson CA, Roper TJ *et al.*: **Characterization and transcription of arsenic respiration and resistance genes during in situ uranium bioremediation**. *ISME J* 2012 http://dx.doi.org/10.1038/ismej.2012.109.

84. Lovley DR, Nevin KP: **A shift in the current: new applications and concepts for microbe-electrode electron exchange**. *Curr Opin Biotechnol* 2011, **22**:441-448.

85. • Beazley MJ, Martinez RJ, Webb SM, Sobecky PA, Taillefert M: **The effect of pH and natural microbial phosphatase activity on the speciation of uranium in subsurface soils**. *Geochim Cosmochim Acta* 2011, **75**:5648-5663.
Authors present an alternative to the process of uranium bioreduction based on sequestration of U(VI) in an oxidized state, which ameliorates concern for re-oxidation of the immobilized end-product.