

Abstracts of the papers

enclosed with the application for Professor of Assoc. Prof. Maxim Boyanov

1. J.L. Goff, Y. Wang, **M.I. Boyanov**, Q. Yu, K.M. Kemner, J.B. Fein, N. Yee, "Tellurite Adsorption onto Bacterial Surfaces", *Environ.Sci.Tech.* 55,15, 10378-10386 (2021) DOI: 10.1021/acs.est.1c01001

Tellurium is an emerging contaminant and its chemical transformation in the environment is strongly influenced by microbial interactions. In this study, we investigated the adsorption of tellurite [Te(IV), TeO₃²⁻] onto the common soil bacterium *Bacillus subtilis*. Thiol-blocking experiments were carried out to investigate the role of cell surface sulfhydryl sites in Te(IV) binding, and X-ray absorption spectroscopy (EXAFS) was performed to determine the chemical speciation of the adsorbed Te(IV). The data indicate that Te(IV) reacts with sulfhydryl functional groups in the extracellular polymeric substances (EPS) produced by *B. subtilis*. Upon binding to sulfhydryl sites in the EPS, Te(IV) changes from Te-O bonds to Te-S coordination. Additional characterization of the *B. subtilis* EPS showed that it contains thiol-rich proteins. Removal of the proteinaceous EPS dramatically decreased Te(IV) adsorption and the sulfhydryl surface site concentrations. These results indicate that sulfhydryl-containing proteins in EPS play a key role in Te(IV) adsorption onto bacterial surfaces.

2. L. Zhang, Y. Chen, Q. Xia, K.M. Kemner, Y. Shen, E.J. O'Loughlin, Z. Pan, Q. Wang, Z. Wang, Y. Huang, H. Dong, **M.I. Boyanov**. "Combined Effects of Fe(III)-bearing Clay Minerals and Organic Ligands on U(VI) Bioreduction and U(IV) Speciation". *Environ.Sci.Technol.* 55, 9, 5929–5938 (2021). doi.org/10.1021/acs.est.0c08645

Reduction of U(VI) to U(IV) drastically reduces its solubility and has been proposed as a method for remediation of uranium contamination. However, much is still unknown about the kinetics, mechanisms, and products of U(VI) bioreduction in complex systems. In this study, U(VI) bioreduction experiments were conducted with *Shewanella putrefaciens* strain CN32 in the presence of clay minerals and two organic ligands, citrate and EDTA. In reactors with U and Fe(III)-clay minerals, the rate of U(VI) bioreduction was enhanced due to the presence of ligands, likely because soluble Fe³⁺- and Fe²⁺-ligand complexes served as electron shuttles. In the presence of citrate, bioreduced U(IV) formed a soluble U(IV)-citrate complex, in experiments with either Fe-rich or Fe-poor clay minerals. In the presence of EDTA, U(IV) occurred as a soluble U(IV)-EDTA complex in Fe-poor montmorillonite experiments. However, U(IV) remained associated with the solid phase in Fe-rich nontronite experiments through the formation of a ternary U(IV)-EDTA-surface complex, as suggested by the EXAFS analysis. Our study indicates that organic ligands and Fe(III)-bearing clays can significantly affect the microbial reduction of U(VI) and the stability of the resulting U(IV) phase.

3. B. Ham, J-S. Kwon, **M.I. Boyanov**, E.J. O'Loughlin, K.M. Kemner, M-J. Kwon, "Geochemical and microbial characteristics of seepage water and mineral precipitates in a radwaste disposal facility affected by seawater intrusion and high alkalinity", *Journal of Environmental Management* 285, 112087 (2021)

The construction of an underground facility can dramatically change the quality, flow direction, and level of groundwater. It may also impact subsurface microbial composition and activity. Groundwater quality was monitored over eight years in two observational wells near an underground disposal facility on the east coast of South Korea. The results showed dramatic increases in dissolved ions such as O₂, Na, Ca, Mg, and SO₄ during facility construction. Seepage water samples downgradient from the silos and tunnels, and precipitates deposited along the seepage water flow path were collected to determine the impact inside the disposal facility. X-ray analysis (powder X-ray diffraction (pXRD) and X-ray absorption fine structure (XAFS)) were used to characterize the mineral precipitates. Microbial community composition was determined by 16S rRNA gene sequencing. The seepage water composition was of two types: Ca-Cl and Ca-Na-HCO₃. The ratio of Cl and δ¹⁸O showed that the Ca-Cl type seepage water was influenced by groundwater mixed with seawater ranging from 2.7% to 15.1%. Various sulfate-reducing bacteria were identified in the Ca-Cl type seepage water, exhibiting relatively high sulfate content from seawater intrusion. Samples from the Ca-Na-HCO₃ type seepage water had an extremely high pH (>10) and abundance of Hydrogenophaga. The precipitates observed along the flow path of the seepage water included calcite, ferrihydrite, green rust, and siderite, depending on seepage water chemistry and microbial activity. This study suggests that the construction of underground structures creates distinct, localized geochemical conditions (e.g., high alkalinity, high salinity, and oxic conditions), which may impact microbial communities. These biogeochemical changes may have undesirable large-scale impacts such as water pump clogging. An understanding of the process and long-term monitoring are essential to assess the safety of underground facilities.

4. Y. Dong, R. Sanford, **M. Boyanov**, K. Kemner, T. Flynn, E. O'Loughlin, S. George, K. Fouke. B. Fouke. "Controls on Iron Reduction and Biomineralization over Broad Environmental Conditions as Suggested by the Firmicutes *Orenia metallireducens* Strain Z6", *Environ.Sci.Tech* 54, 10128-10140 (2020)

The reduction of ferric oxide minerals is a ubiquitous biogeochemical process that occurs over a broad range of geochemical conditions and is driven by a diverse array of microorganisms. This process results in the formation of a variety of ferrous secondary minerals, many of which are highly reactive with organic and metal contaminants and therefore important for understanding their fate and transport in the environment. Here we examine the formation of secondary iron minerals over a broad range of environmental conditions by *Orenia metallireducens* strain Z6, an member of the Firmicutes capable of reducing metal oxides. We tested 19 distinct geochemical conditions with differing pH (6.5-8.5), temperature (22-50 °C), salinity (2-20% NaCl), anion availability (phosphate and sulfate), electron shuttle availability (anthraquinone-2,6-disulfonate (AQDS)), and ferric iron oxide mineralogy [2-line ferrihydrite [Fe(OH)₃],

lepidocrocite (α -FeOOH), goethite (β -FeOOH), hematite (Fe_2O_3), and magnetite (Fe_3O_4)]. In these experiments, the rate (kint -0.186 to 1.702 $\text{mmol}\cdot\text{L}^{-1}\cdot\text{day}^{-1}$) and magnitude (between 6.3-83.7% of the initial Fe(III) was reduced) of iron reduction by *O. metallireducens* differed significantly. Using X-ray absorption and scattering techniques, we observed that siderite (FeCO_3), vivianite [$\text{Fe}_3(\text{PO}_4)_2$] and green rust were the most common secondary minerals formed, while $\text{Fe}(\text{OH})_2$, hematite and ferrihydrite were formed under limited conditions. Unlike the previous findings, we could not attribute the distribution and relative abundance of these biogenic minerals to the rate or magnitude of microbial Fe(II) production alone. Instead, we found a bifurcation point based on the extent to which Fe(II) sorbed to the remaining Fe(III) minerals that was critical in determining the dissolution/reprecipitation reactions that led to the formation secondary minerals.

5. J. Dickson, N.A. Conroy, B.A. Powell, J.C. Seaman, **M.I. Boyanov**, K.M. Kemner, D.I. Kaplan, "Surfactant-modified siliceous zeolite Y for pertechnetate remediation". *Chemical Engineering Journal* 402, 126268 (2020)

Separation of pertechnetate ($^{99}\text{TcO}_4^-$) from contaminated groundwater and nuclear waste streams remains a technical challenge for the long-term management of $^{99}\text{TcO}_4^-$ releases to the environment. Herein, a siliceous zeolite mineral (Faujasite) was modified with cationic surfactant (hexadecyltrimethylammonium bromide – HDTMA) and subsequently used to sequester TcO_4^- from acidic aqueous solution containing competing ions. Pristine and modified zeolite phase(s) were characterized with X-ray diffraction, X-ray absorption spectroscopy (XAS), sorption isotherms, zeta potential, and BET surface area measurement. Measured sorption capacity of the modified zeolite phases was 337 mg/g, which was in good agreement with modelled estimates, 357 mg/g. The Langmuir and the pseudo-second-order models adequately described the sorption of TcO_4^- on the modified zeolite phases. Kinetic data suggest that liquid diffusion and intraparticle diffusion were the rate-limiting steps governing TcO_4^- sorption. Thermodynamic data revealed that TcO_4^- adsorption was spontaneous and exothermic. Analyses of the XAS spectra data revealed that ReO_4^- , a chemical analogue for TcO_4^- , was associated with the modified zeolite phase(s) as an inner-sphere complex of ReO_4^- and there was no evidence of the reduced form of Re(IV). The modified zeolite had fast sorption kinetics, significant sorption capacity, and robust uptake performance in acidic conditions (pH 3-5) and in the presence of high concentrations of competing anions. Furthermore, an important attribute of this remediation technology is that it sequesters the oxidized form of Tc (TcO_4^-), that exists naturally in most surface environments and waste streams and it provides an effective alternative to the existing reductive precipitation technologies that have limited long-term environmental applications

6. Crawford B., Strobbia P., Wang H-N., Zentella-Gomez R., **Boyanov M.I.**, Pei Z-M., Sun T-P., Kemner K. M., Vo-Dinh T. "Plasmonic Nanoprobes for in Vivo Multimodal Sensing and Bioimaging of MicroRNA within Plants", *ACS Applied Materials & Interfaces*, 11 (8) 7743–7754 (2019)

Monitoring gene expression within whole plants is critical for many applications ranging from plant biology to agricultural biotechnology and biofuel development; however, no method currently exists for in vivo monitoring of genomic targets in plant systems without requiring sample extraction. Herein, we report a unique multimodal method based on plasmonic nanoprobe capable of in vivo imaging and biosensing of microRNA biotargets within whole plant leaves by integrating three different and complementary techniques: surface-enhanced Raman scattering (SERS), X-ray fluorescence (XRF), and plasmonics-enhanced two-photon luminescence (TPL). The method developed uses plasmonic nanostars, which not only provide large Raman signal enhancement but also allow for localization and quantification by XRF and plasmonics-enhanced TPL, owing to gold content and high two-photon luminescence cross sections. Our method uses inverse molecular sentinel nanoprobe for SERS bioimaging of microRNA within *Arabidopsis thaliana* leaves to provide a dynamic SERS map of detected microRNA targets while also quantifying nanoprobe concentrations using XRF and TPL. The nanoprobe were observed to occupy the intercellular spaces upon infiltration into the leaf tissues. This report lays the foundation for the use of plasmonic nanoprobe for in vivo functional imaging of nucleic acid biotargets in whole plants, a tool that will revolutionize bioengineering research by allowing the study of these biotargets with previously unmet spatial and temporal resolution, 200 μm and 30 min, respectively.

7. Q. Yu, **M.I. Boyanov**, J. Liu, K. M. Kemner, J. Fein. "Adsorption of selenite onto *Bacillus subtilis*: the overlooked role of cell envelope sulfhydryl sites in the microbial conversion of Se(IV)", *Environ.Sci.Tech* 52, 18, 10400-10407 (2018)

Microbial activities play a central role in the global cycling of selenium. Microorganisms can reduce, methylate and assimilate Se, controlling the transport and fate of Se in the environment. However, the mechanisms controlling these microbial activities are still poorly understood. In particular, it is unknown how the negatively-charged Se(IV) and Se(VI) oxyanions that dominate the aqueous Se speciation in oxidizing environments bind to negatively-charged microbial cell surfaces in order to become bioavailable. Here, we show that the adsorption of selenite onto *Bacillus subtilis* bacterial cells is controlled by cell envelope sulfhydryl sites. Once adsorbed onto the bacteria, selenite is reduced, and forms reduced organo-Se compounds (e.g., R1S-Se-SR2). Because sulfhydryl sites are present within cell envelopes of a wide range of bacterial species, sulfhydryl-controlled adsorption of selenite likely represents a general mechanism adopted by bacteria to make selenite bioavailable. Therefore, sulfhydryl binding of selenite likely occurs in a wide range of oxidized Se-bearing environments, and because it is followed by microbial conversion of selenite to other Se species, the process represents a crucial step in the global cycling of Se.

8. S. Yan, **M.I. Boyanov**, B. Mishra, K.M. Kemner, E.J. O'Loughlin. "U^{VI} Reduction by Biogenic and Abiotic Hydroxycarbonate Green Rusts: Kinetics and Mechanism" *Environ.Sci.Tech.* 52 (2018) 4601–4609

Green rusts (GRs) are redox active FeII-FeIII minerals that form in the environment via various biotic and abiotic processes. Although both biogenic (BioGR) and abiotic (ChemGR) GRs have been shown to reduce UVI, the dynamics of the transformations and the speciation and stability of the resulting UIV phases are poorly understood. We used carbonate extraction and XAFS spectroscopy to investigate the products of UVI reduction by BioGR and ChemGR. The results show that both GRs can rapidly remove UVI from synthetic groundwater via reduction to UIV. The initial products in the ChemGR system are solids-associated UIV-carbonate complexes that gradually transform to nanocrystalline uraninite over time, leading to a decrease in the proportion of carbonate-extractable U from ~95% to ~10%. In contrast, solid-phase UIV atoms in the BioGR system remain relatively extractable, non-uraninite UIV species over the same reaction period. The presence of calcium and carbonate in groundwater significantly increase the extractability of UIV in the BioGR system. These data provide new insights into the transformations of U under anoxic conditions in groundwater that contains calcium and carbonate, and have major implications for predicting uranium stability within redox dynamic environments and designing approaches for the remediation of uranium-contaminated groundwater.

9. M.-J.Kwon, **M.I.Boyanov**, J.-S. Yang, S.Lee, Y.-H.Hwang, J.-Y. Lee, B. Mishra, K.M.Kemner, "Transformation of zinc-concentrate in surface and subsurface environments: Implications for assessing zinc mobility/toxicity and choosing an optimal remediation strategy", *Environmental Pollution*, 226 (2017) 346-355.

Zn contamination in surface and subsurface environments is a serious threat to ecosystems and to public health, so understanding its fate and transport is critical to evaluating environmental risk and to developing remediation strategies. The geochemical and mineralogical characteristics of contaminated sediments in the vicinity of a Zn ore transportation route in South Korea were investigated using a variety of analytical techniques (sequential extraction, XRF, XRD, SEM, and XAFS). The results show that Zn concentrate (ZnS) imported from overseas was directly dispersed to the roadside and surrounding areas including rice paddy soils, and subsequently moved to the subsurface as a result of physical and chemical weathering. Zn minerals identified in the contaminated sites included zinc sulfide, zinc carbonate, and O-coordinated zinc minerals. The zinc concentrate transformed mainly into O-coordinated zinc as well as zinc species bound to Fe/Mn oxides, as confirmed by XAFS and sequential extraction. For the purpose of developing a soil washing remediation process, the contaminated samples were extracted with dilute acids (0.1 and 1.0 N). The extraction efficiency increased in the order $H_3PO_4 < HNO_3 < H_2SO_4 < HCl$, as well as with increased proportion of O-coordinated Zn and decreased proportion of ZnS in the sediment. This study demonstrates that a thorough understanding of the metal speciation and mineralogy of contaminated sediments is essential for tracking the source of toxic metals and for developing an optimal remediation strategy using soil washing.

10. M.I.Boyanov, D.E.Latta,M.M.Scherer, E.J.O'Loughlin, K.M.Kemner, "Surface area effects on the reduction of U^{VI} in the presence of synthetic montmorillonite", *Chemical Geology*, 464 (2017) 110-117

The redox transformations that affect the environmental mobility of metal or radionuclide contaminants typically take place in the presence of mineral or biological surfaces. Adsorption can alter the speciation and free energy of a dissolved ion and can thus change the occurrence, rate, or products of redox reactions relative to those expected in homogenous solution. Here, we investigated the effect of SYn-1, a redox-inactive synthetic montmorillonite clay mineral, on the reduction of UVI by dihydroanthraquinone-2,6 disulfonate (AH2QDS, the reduced form of the soluble electron shuttle AQDS). We varied the surface:U ratio in a circumneutral bicarbonate solution and measured the valence and atomic coordination of U in the solids using x-ray absorption spectroscopy (XANES and EXAFS). The spectra show that UIV was the predominant product both in the presence or absence of the clay mineral, indicating that adsorption of UVI to clay mineral surface sites does not affect its ability to be reduced by AH2QDS. In the absence of the clay mineral and at low-to-intermediate surface:U ratios the predominant UIV product was nanoparticulate uraninite, UO₂. At the highest surface:U ratio tested (100 g/L clay mineral and 50 μM U) we observe a decrease in the proportion of uraninite down to 50%, with the remaining UIV present as adsorbed, non-uraninite species. These results are similar to previous findings with magnetite and rutile, where adsorbed UIV were the predominant species below a specific surface coverage and U in excess of this coverage was precipitated as uraninite. The threshold coverage determined here for the SYn-1 clay mineral is 10-100x lower than that determined for magnetite and rutile, suggesting that clay mineral surfaces may be less important than metal oxides for stabilizing non-uraninite UIV species in natural sediments.

11. Y. Dong, R. Sanford, M. Boyanov, K. Kemner, T. Flynn, E. O'Loughlin, R. Locke, J. Weber, S. Egan, R. Mackie, I. Cann, B. Fouke. "Orenia metallireducens sp. nov. Strain Z6, a novel metal-reducing member of the phylum *Firmicutes* from the deep subsurface" *Applied and Environmental Microbiology*, 82, 21 (2016) 6440-6453

A novel halophilic and metal-reducing bacterium, *Orenia metallireducens* strain Z6, was isolated from briny groundwater extracted from a 2.02 km-deep borehole in the Illinois Basin, IL. This organism shared 96% 16S rRNA gene similarity with *Orenia marismortui* but demonstrated physiological properties previously unknown for this genus. In addition to exhibiting a fermentative metabolism typical of the genus *Orenia*, strain Z6 reduces various metal oxides [Fe(III), Mn(IV), Co(III), and Cr(VI)], using H₂ as the electron donor. Strain Z6 actively reduced ferrihydrite over broad ranges of pH (6 to 9.6), salinity (0.4 to 3.5M NaCl), and temperature (20 to 60°C). At pH 6.5, strain Z6 also reduced more crystalline iron oxides, such as lepidocrocite (-FeOOH), goethite (—————FeOOH), and hematite (—————Fe₂O₃). Analysis of X-ray absorption fine structure (XAFS) following Fe(III) reduction by strain Z6 revealed spectra from ferrous secondary mineral phases consistent with the precipitation of vivianite [Fe₃(PO₄)₂] and

siderite (FeCO₃). The draft genome assembled for strain Z6 is 3.47 Mb in size and contains 3,269 protein-coding genes. Unlike the well-understood iron-reducing *Shewanella* and *Geobacter* species, this organism lacks the c-type cytochromes for typical Fe(III) reduction. Strain Z6 represents the first bacterial species in the genus *Orenia* (order Halanaerobiales) reported to reduce ferric iron minerals and other metal oxides. This microbe expands both the phylogenetic and physiological scopes of iron-reducing microorganisms known to inhabit the deep subsurface and suggests new mechanisms for microbial iron reduction. These distinctions from other *Orenia* spp. support the designation of strain Z6 as a new species, *Orenia metallireducens* sp. nov.

12. M.-J. Kwon, S. Leeb, G. Leec, B. Hama, **M. I. Boyanov**, K. Kemner, E. O'Loughlin, J.-S. Yang. "Geochemical Characteristics and Microbial Community Compositions in Toxic Metal-rich Sediments Contaminated from Au-Ag Mine Tailings", *J. Hazardous Materials* 296 (2015) 147–157

The effects of extreme geochemical conditions on microbial community composition were investigated using two distinct sets of sediment samples collected near weathered mine tailings. One set (SCH) showed extraordinary geochemical characteristics: As (6.7-11.5%), Pb (1.5-2.1%), Zn (0.1-0.2%), and pH (3.1-3.5). The other set (SCL) had As (0.3-1.2%), Pb (0.02-0.22%), and Zn (0.01-0.02%) at pH of 2.5-3.1. The bacterial communities in SCL were clearly different from those in SCH suggesting extreme geochemical conditions affected microbial community distribution even on a small spatial scale. The clones identified in SCL were closely related to acidophilic bacteria within the taxa *Acidobacterium* (18%), *Acidomicrobinae* (14%), and *Leptospirillum* (10%). Most clones in SCH were closely related to *Methylobacterium* (79%) and *Ralstonia* (19%), both of which are well-known metal-resistant bacteria. Although total As was extremely high, over 95% of it was in the form of scorodite (FeAsO₄·2H₂O). Acid extractable As was only ~118 and ~14 ppm in SCH and SCL, respectively, which is not known to be toxic to bacteria. Meanwhile, acid extractable Pb and Zn in SCH were above concentrations toxic to bacteria. Because As was present in an oxidized and stable form it is likely that Pb and/or Zn released from the sediment were mainly responsible for the differences in microbial community structure.

13. D. E. Latta, **M. I. Boyanov**, K. M. Kemner, E. O'Loughlin, M. Scherer. "Reaction of U(VI) with green rusts: Effect of interlayer anion", *Current Inorganic Chemistry* 5, 156-168 (2015)

Green rusts are widely recognized as an important metastable intermediate phase in Fe biogeochemical cycling and Fe metal corrosion and are strong reductants capable of reducing a wide-range of contaminants. Here we investigate the effect of interlayer anion on the reaction of green rusts with hexavalent uranium (U(VI)). We react three synthetic green rusts, including carbonate, sulfate, and chloride green rust, as well as pyroaurite, a redox-inactive Mg(II)-Fe(III) structural analog of carbonate green rust with U(VI). The majority of U(VI) (> 80%) was removed from solution in about an hour at pH 8.0 in 0.1 M N-Tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid (TAPS) buffer. Similar kinetics of

U(VI) uptake on green rusts and pyroaurite suggest that the observed uptake kinetics reflect an adsorption step rather than reduction of U(VI) by structural Fe(II) in the green rusts. X-ray absorption spectroscopy (XAS) of the green rust solids indicates significant reduction of U(VI) to U(IV) occurred for all three green rusts, with complete reduction observed for sulfate and chloride green rust and varied extents of reduction (34 to 100%) observed for carbonate green rust depending on the solution conditions. No reduction of U(VI) was observed in the presence of pyroaurite, consistent with the absence of Fe(II) in the pyroaurite structure. The decreased amount of U(VI) reduction observed for carbonate green rust in TAPS buffer may be due to modification of the redox properties of U(VI) or green rust by complexation with TAPS buffer. XAS results indicate that U(VI) was reduced to U(IV) and was present as a monomeric-type U(IV) species in the presence of TAPS buffer. In deionized water, however, carbonate green rust reduced U(VI) to nanoparticulate UO₂. Green rusts may be an important phase in the fate and transport of U(VI) in the contaminated subsurface, or under conditions where it forms on corroding U-bearing waste containers.

14. J.-K. Choe, **M.I. Boyanov**, J. Liu, K.Kemner, C.Werth, T. Strathmann, "X-ray spectroscopic characterization of immobilized rhenium species in hydrated rhenium-palladium bimetallic catalysts used for perchlorate water treatment", *J.Phys.Chem.C*, 118(22), 11666-11676 (2014)

Carbon-supported rhenium-palladium catalysts (Re-Pd/C) effectively transform aqueous perchlorate, a widespread drinking water pollutant, via chemical reduction using hydrogen as electron donor at ambient temperature and pressure. Previous work demonstrated that catalyst activity and stability are heavily dependent on solution composition and Re content in the catalyst. This study relates these parameters to changes in the speciation and molecular structure of Re immobilized on the catalyst. Using X-ray characterization techniques, we show that Re is immobilized as Re(VII) under oxic solution conditions, but transforms to a mixture of reduced, O-coordinated Re species under reducing solution conditions induced by H₂ sparging. Under oxic solution conditions, extended x-ray absorption fine structure (EXAFS) analysis showed that the immobilized Re(VII) species is indistinguishable from the dissolved tetrahedral perrhenate (ReO₄⁻) anion, suggesting outer-sphere adsorption to the catalyst surface. Under reducing solution conditions, two Re species were identified. At low Re loading (□ 1 wt%), monomeric Re(I) species form in direct contact with Pd nanoclusters. With increased Re loading, speciation gradually shifts to oxidic Re(V) clusters. The identified Re structures support a revised mechanism for catalytic reduction of ClO₄⁻ involving oxygen atom transfer reactions between odd-valence oxorhenium species and the oxyanion (Re oxidation steps) and atomic hydrogen species (Re reduction steps) formed by Pd-catalyzed dissociation of H₂.

15. D. E. Latta, B. Mishra, R.E. Cook, K. M. Kemner, **M. I. Boyanov**, “Stable U(IV) complexes form at high-affinity mineral surface sites”, *Environ.Sci.Tech.*, 48 (3), 1683–1691 (2014)

Uranium (U) poses a significant contamination hazard to soils, sediments, and groundwater due to its extensive use for energy production. Despite advances in modeling the risks of this toxic and radioactive element, lack of information about the mechanisms controlling U transport hinders further improvements, particularly in reducing environments where UIV predominates. Here, we establish that mineral surfaces can stabilize the majority of U as adsorbed UIV species following reduction of UVI. Using x-ray absorption spectroscopy and electron imaging analysis, we find that at low surface loading UIV forms inner-sphere complexes with two metal oxides, TiO₂ (rutile) and Fe₃O₄ (magnetite) (at <1.3 U nm⁻² and <0.037 U nm⁻², respectively). The uraninite (UO₂) form of UIV predominates only at higher surface loading. UIV -TiO₂ complexes remain stable for at least 12 months and UIV -Fe₃O₄ complexes remain stable for at least 4 months under anoxic conditions. Adsorbed UIV results from UVI reduction by FeII or by the reduced electron shuttle AH₂QDS, suggesting that both abiotic and biotic reduction pathways can produce stable UIV -mineral complexes in the subsurface. The observed control of high-affinity mineral surface sites on UIV speciation helps explain the presence of non-uraninite UIV in sediments and has important implications for U transport modeling.

16. D. B. Watson, W-M. Wu, T. Mehlhorn, G. Tang, J. Earles, K. Lowe, T. M. Gihring, G. Zhang, F. Zhang, J. Phillips, **M. Boyanov**, B. Spalding, C. Schadt, K. M. Kemner, C. S. Criddle, P. M. Jardine, S. C. Brooks, “In situ Bioremediation of Uranium with Emulsified Vegetable Oil as the Electron Donor”, *Environ. Sci. Technol.*, 47 (12), 6440–6448 (2013)

A field test with a one-time emulsified vegetable oil (EVO) injection was conducted in a highly permeable gravelly layer in the uranium contaminated subsurface containing (mM) U, 0.0055; Ca, 2.98; NO₃⁻, 0.11; HCO₃⁻, 5.07; and SO₄²⁻, 1.23. Comparison of bromide and EVO migration and distribution indicated that a majority of the injected EVO was retained in the subsurface. Nitrate, uranium, and sulfate were sequentially removed from the groundwater within 1-2 weeks, accompanied by an increase of acetate, Mn, Fe, and methane concentration. Due to the slow release and degradation of the retained EVO with time, reducing conditions were sustained for approximately one year and daily U discharge to a surface water receptor, located approximately 50 m from the injection wells, decreased by 80% within 100 days. Total U discharge was reduced by 50% over the one year period. Reduction of U(VI) to U(IV) was confirmed by synchrotron analysis of recovered aquifer solids. Oxidants (e.g, dissolved oxygen, nitrate) flowing in from upgradient appeared to re-oxidize and re-mobilize uranium after the EVO was exhausted as evidenced by a transient increase of U concentration above the upgradient and pre-injection values and then stabilized. EVO injection can sustain uranium bioreduction/immobilization in Ca and bicarbonate-containing aquifer with seasonal injection to effectively decrease U discharge.

17. X. Rui, M-J. Kwon, E.J.O'Loughlin, S-D. Cheatam, J. Fein, B. Bunker, K. Kemner, **M. I. Boyanov**, "Bioreduction of hydrogen uranyl phosphate: mechanisms and U(IV) products", *Environ. Sci. Technol.*, 47 (11), 5668–5678 (2013)

The mobility of uranium (U) in subsurface environments is controlled by interrelated adsorption, redox, and precipitation reactions. Previous work demonstrated the formation of nanometer-sized hydrogen uranyl phosphate (abbreviated as HUP) crystals on the cell walls of *Bacillus subtilis*, a non-UVI-reducing, Gram-positive bacterium. The current study examined the reduction of this biogenic, cell-associated HUP mineral by three dissimilatory metal-reducing bacteria: *Anaeromyxobacter dehalogenans* strain K, *Geobacter sulfurreducens* strain PCA, and *Shewanella putrefaciens* strain CN-32, and compared it to the bioreduction of abiotically formed and freely suspended HUP of larger particle size. Uranium speciation in the solid phase was followed over a 10- to 20-day reaction period by x-ray absorption fine structure spectroscopy (XANES and EXAFS) and showed varying extents of UVI reduction to UIV. The reduction extent of the same mass of HUP to UIV was consistently greater with the biogenic than with the abiotic material under the same experimental conditions. A greater extent of HUP reduction was observed in the presence of bicarbonate in solution, whereas a decreased extent of HUP reduction was observed with the addition of dissolved phosphate. These results indicate that the extent of UVI reduction is controlled by dissolution of the HUP phase, suggesting that the metal-reducing bacteria transfer electrons to the dissolved or bacterially adsorbed UVI species formed after HUP dissolution, rather than to solid-phase UVI in the HUP mineral. Interestingly, the bioreduced UIV atoms were not immediately coordinated to other UIV atoms (as in uraninite, UO₂), but were similar in structure to the phosphate-complexed UIV species found in ningyoite [CaU(PO₄)₂·H₂O]. This indicates a strong control by phosphate of the speciation of bioreduced UIV, expressed as inhibition of the typical formation of uraninite under phosphate-free conditions.

18. R.S. Renslow, J.T. Babauta, A. Dohnalkova, **M.I. Boyanov**, K.M. Kemner, P.D. Majors, J.K. Fredrickson, H. Beyenal, "Metabolic spatial variability in electrode-respiring *Geobacter sulfurreducens* biofilms", *Energy Environ. Sci.*, 6, 1827-1836 (2013)

In this study, we quantified electron donor profiles and biofilm structure in electrode-respiring *Geobacter sulfurreducens* biofilms to address the limiting steps in electron transfer processes. Using an electrochemical-NMR biofilm reactor, we quantified the electron transfer rate and depth profiles of acetate and porosity. Cells near the top of the biofilms consumed acetate and were metabolically active; however, acetate concentration decreased to below detection within 100 microns from the top of the biofilms. Additionally, porosity in the biofilms fell below 10% near the electrode surface, exacerbating exclusion of acetate from the lower regions. The dense biofilm matrix in the acetate-depleted zone acted as an electrical conduit to pass electrons generated at the top of the biofilm to the electrode. To verify the distribution of cell metabolic activity, we used uranium as a redox-active probe for localizing electron transfer activity and X-ray absorption spectroscopy to determine the

uranium oxidation state. Cells near the top of the biofilm reduced UVI more actively than the cells near the base. High-resolution TEM images showed intact, healthy cells near the top and plasmolyzed cells near the base. Contrary to models proposed in the literature, which hypothesize that cells nearest to the electrode surface are the most metabolically active due to a lower electron transfer resistance, our results suggest that electrical resistance through the biofilm is not restricting long-range electron transfer. Cells far from the electrode can respire across metabolically inactive cells, taking advantage of their extracellular infrastructure for electron transfer produced during the initial biofilm formation.

19. C. Dimkpa, J. McLean, D. Latta, E. Manangon, D. Britt, W. Johnson, **M. Boyanov**, A. Anderson, "CuO and ZnO nanoparticles: phytotoxicity, metal speciation, and induction of oxidative stress in sand-grown wheat", *Journal of Nanoparticle Research*, 14:1125-1139 (2012)

Metal oxide nanoparticles (NPs) are reported to impact plant growth in hydroponic systems. This study describes the impact of commercial CuO (150 nm) and ZnO (100 nm) NPs on wheat (*Triticum aestivum*) grown in a solid matrix, sand. The NPs contained both metallic and non-metallic impurities to different extents. Dynamic light scattering and atomic force microscopy (AFM) assessments confirmed aggregation of the NPs to submicron sizes. AFM showed transformation of ZnO NPs from initial rhomboid shapes in water to elongated rods in the aqueous phase of the sand matrix. Solubilization of metals occurred in the sand at similar rates from CuO or ZnO NPs as their bulk equivalents. Amendment of the sand with 500 mg Cu and Zn/kg sand from the NPs significantly ($p = 0.05$) reduced root growth, but only CuO NPs impaired shoot growth; growth reductions were less with the bulk amendments. Dissolved Cu from CuO NPs contributed to their phytotoxicity but Zn release did not account for the changes in plant growth. Bioaccumulation of Cu, mainly as CuO and Cu(I)-sulfur complexes, and Zn as Zn-phosphate was detected in the shoots of NP-challenged plants. Total Cu and Zn levels in shoot were similar whether NP or bulk materials were used. Oxidative stress in the NP-treated plants was evidenced by increased lipid peroxidation and oxidized glutathione in roots and decreased chlorophyll content in shoots; higher peroxidase and catalase activities were present in roots. These findings correlate with the NPs causing increased production of reactive oxygen species. The accumulation of Cu and Zn from NPs into edible plants has relevance to the food chain.

20. B. Ahmed, B. Cao, B. Mishra, **M. Boyanov**, K. Kemner, J. Fredrickson, H. Beyenal, "Immobilization of U(VI) from oxic groundwater by Hanford 300 Area sediments and effects of Columbia River water", *Water Research*, 46(13): 3989-3998 (2012)

The U.S. Department of Energy Hanford 300 Area (300A) site experiences periodic hydrologic influences from the nearby Columbia River as a result of changing river stage, which causes changes in groundwater elevation, flow direction and water chemistry. An important question is the extent to which the mixing of Columbia River water and groundwater impacts the speciation and mobility of uranium (U). In this study, we designed experiments to mimic interactions among U, oxic groundwater or Columbia River water, and 300A sediments in the subsurface

environment of Hanford 300A. The goals were to investigate mechanisms of: 1) U immobilization in 300A sediments under bulk oxic conditions and 2) U remobilization from U-immobilized 300A sediments exposed to oxic Columbia River water. Initially, 300A sediments in column reactors were fed with U(VI)-containing oxic 1) synthetic groundwater (SGW), 2) organic-amended SGW (OA-SGW), and 3) de-ionized (DI) water to immobilize U. After that, the sediments were exposed to oxic Columbia River water for U remobilization studies. The results reveal that U was immobilized by 300A sediments predominantly through reduction (80-85%) when the column reactor was fed with oxic OA-SGW. However, U was immobilized by 300A sediments through adsorption (100%) when the column reactors were fed with oxic SGW or DI water. The reduced U in the 300A sediments fed with OA-SGW was relatively resistant to remobilization by oxic Columbia River water. Oxic Columbia River water resulted in U remobilization (~7%) through desorption, and most of the U that remained in the 300A sediments fed with OA-SGW (~93%) was in the form of uraninite nanoparticles. These results reveal that: 1) the reductive immobilization of U through OA-SGW stimulation of indigenous 300A sediment micro-organisms may be viable in the relatively oxic Hanford 300A subsurface environments and 2) with the intrusion of Columbia River water, desorption may be the primary process resulting in U remobilization from OA-SGW-stimulated 300A sediments at the subsurface of the Hanford 300A site.

21. H.-B. Jung, **M. Boyanov**, H. Konishi, B. Mishra, K. Kemner, E. Roden, H. Xu, "Redox Behavior of Uranium at the Nanoporous Aluminum Oxide-Water Interface: Implication for Uranium Remediation", *Environ. Sci. Technol* 46(13):7301-7309, (2012)

Sorption-desorption experiments demonstrated that mesoporous and nanoporous aluminum oxide had a significantly higher chemical affinity for U(VI) compared to non-nanoporous alpha-alumina. Specifically, the majority (ca. 80-90%) U(VI) presorbed to mesoporous and nanoporous alumina could not be released by extended (2 weeks) extraction with 50 mM NaHCO₃. The extent of reduction of U(VI) presorbed to aluminum oxides was semi-quantitatively estimated by comparing the percentages of uranium desorbed by anoxic sodium bicarbonate between AH2DS-reacted and unreacted control samples. X-ray absorption spectroscopy confirmed that U(VI) presorbed to non-nanoporous alumina was rapidly and completely reduced to nanoparticulate uraninite by AH2DS, whereas reduction of U(VI) presorbed to nanoporous alumina was slow and incomplete (0±5% reduction after 1 week). The observed nanopore size-dependent redox behavior of U has important implications in developing efficient remediation techniques for the subsurface uranium contamination because the efficiency of in-situ bioremediation depends on how effectively and rapidly U(VI) bound to sediment or soil can be converted to an immobile phase.

22. D. Latta, **M. Boyanov**, K. Kemner, E. O'Loughlin, M. Scherer, "Abiotic reduction of uranium by Fe(II) in soil", *Applied Geochemistry*, 27, 8, 1512-1524 (2012)

Structural Fe(II) has been shown to reduce several oxidized environmental contaminants, including NO₃, chlorinated solvents, Cr(VI), and U(VI). Studies investigating reduction of U(VI) by soils and sediments, however, suggest that abiotic reduction of U(VI) by Fe(II) is not

significant, and that direct enzymatic reduction of U(VI) by metal-reducing bacteria is required for U(VI) immobilization as U(IV). Here evidence is presented for abiotic reduction and immobilization of U(VI) by structural Fe(II) in a redoximorphic soil collected from a hillside spring in Iowa. Oxidation of Fe(II) in the soil after reaction with U(VI) was demonstrated by Mössbauer spectroscopy and reduction of U(VI) by the pasteurized soil using U LIII-edge X-ray absorption spectroscopy (XAS). XAS indicates that both reduced U(IV) and oxidized U(VI) or U(V) are present after U(VI) interaction with the Fe(II) containing soil. The EXAFS data show the presence of a non-uraninite U(IV) phase and evidence of the oxidized U(V) or U(VI) fraction being present as a nonuranyl species. Little U(VI) reduction is observed by soil that has been exposed to air and oxidation of Fe(II) to goethite has occurred. Soil characterization based on chemical extractions, Mössbauer spectroscopy, and Fe K-edge XAS indicate that the majority of Fe(II) in the soil is structural in nature, existing in clay minerals and possibly a green rust-like phase. These data provide compelling evidence for abiotic reduction of U(VI) by structural Fe(II) from soil near Fe-rich oxic–anoxic boundaries in natural environments. The work highlights the potential for abiotic reduction of U(VI) by Fe(II) in reduced, Fe-rich environments.

23. J.-H. Lee, J. Fredrickson, X. Lin, R. Kukkadapu, **M. Boyanov**, K. Kemner, D. Kennedy, B. Bjornstad, A. Konopka, D. Moore, C. Resch, J. Phillips. “Microbial Reductive Transformation of Phyllosilicate Fe(III) and U(VI) in Fluvial Subsurface Sediments”. *Environ. Sci. Technol.*, 46, 3721–3730 (2012)

The microbial reduction of Fe(III) and U(VI) were investigated in shallow aquifer sediments collected from subsurface flood deposits near the Hanford Reach of the Columbia River in Washington State. Increases in 0.5 N HCl-extractable Fe(II) were observed in incubated sediments and ^{57}Fe Mössbauer spectroscopy revealed that Fe(III) associated with phyllosilicates and pyroxene was reduced to Fe(II). Aqueous uranium(VI) concentrations decreased in subsurface sediments incubated in sulfate-containing synthetic groundwater with the rate and extent being greater in sediment amended with organic carbon. X-ray absorption spectroscopy of bioreduced sediments indicated that 67-77% of the U signal was U(VI), probably as an adsorbed species associated with a new or modified reactive mineral phase. Phylotypes within the Deltaproteobacteria were more common in Hanford sediments incubated with U(VI) than without and in U(VI)-free incubations, members of the Clostridiales were dominant with sulfate-reducing phylotypes more common in the sulfate-amended sediments. These results demonstrate the potential for anaerobic reduction phyllosilicate Fe(III) and sulfate in Hanford unconfined aquifer sediments and biotransformations involving reduction and adsorption leading to decreased aqueous U concentrations.

24. H. Nguyen, B. Cao, B. Mishra, **M. Boyanov**, K. Kemner, J. Fredrickson, H. Beyenal, “Microscale geochemical gradients in Hanford 300 Area sediment biofilms and influence of uranium”, *Water Research*, 46, 227-234 (2012)

The presence and importance of microenvironments in the subsurface at contaminated sites were suggested by previous geochemical studies. However, no direct quantitative characterization of the geochemical microenvironments had been reported. We quantitatively

characterized microscale geochemical gradients (dissolved oxygen (DO), H₂, pH, and redox potential) in Hanford 300A subsurface sediment biofilms. Our results revealed significant differences in geochemical parameters across the sediment biofilm/water interface in the presence and absence of U(VI) under oxic and anoxic conditions. While the pH was relatively constant within the sediment biofilm, the redox potential and the DO and H₂ concentrations were heterogeneous at the microscale (<500e1000 mm). We found microenvironments with high DO levels (DO hotspots) when the sediment biofilm was exposed to U(VI). On the other hand, we found hotspots (high concentrations) of H₂ under anoxic conditions both in the presence and in the absence of U(VI). The presence of anoxic microenvironments inside the sediment biofilms suggests that U(VI) reduction proceeds under bulk oxic conditions. To test this, we operated our biofilm reactor under air-saturated conditions in the presence of U(VI) and characterized U speciation in the sediment biofilm. U LIII-edge X-ray absorption spectroscopy (XANES and EXAFS) showed that 80-85% of the U was in the U(IV) valence state.

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

Recent UVI bioreduction studies report on the formation of UIV species that lack the U=O₂=U coordination of uraninite. To determine the controls on the formation of such non-uraninite UIV species, the current work studied the reduction of carbonate-complexed UVI by: 1) five Gram-positive *Desulfitobacterium* strains, 2) the Gram-negative bacteria *Anaeromyxobacter dehalogenans* 2CP-C and *Shewanella putrefaciens* CN32, and 3) chemically reduced 9,10-anthrahydroquinone-2,6-disulfonate (AH₂QDS). Further, the effects of 0.3 mM dissolved phosphate on UIV species formation were explored. Extended x-ray absorption fine structure (EXAFS) analysis demonstrated that the addition of phosphate causes the formation of a non-uraninite, phosphate-complexed UIV species, independent of the biological or abiotic mode of UVI reduction. In phosphate-free medium, UVI reduction by *Desulfitobacterium* spp. and by AH₂QDS resulted in non-uraninite, carbonate-complexed UIV species, whereas reduction by *A. dehalogenans* or *S. putrefaciens* yielded nanoparticulate uraninite. These findings suggest that the Gram-positive *Desulfitobacterium* strains and the Gram-negative species *A. dehalogenans* and *S. putrefaciens* use distinct mechanisms to reduce UIV.