Integrating *In Silico* Modeling and Proteomics into Field-Scale Simulations of Uranium Biogeochemical Reactive Transport

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The Rifle IFRC project is working towards a holistic understanding of the processes, properties, and conditions controlling uranium behavior as indigenous metal-reducing bacteria are stimulated to enzymatically transform aqueous U(VI) to immobile, solid-associated U(IV). A comprehensive modeling framework is used to systematically represent the impact of acetate biostimulation on the microbial community, terminal electron acceptors, and uranium mobility in the context of the Rifle site geology, hydrology, and biogeochemistry. In this case, three-dimensional, variably-saturated flow and biogeochemical reactive transport modeling is used to simulate the impacts of pulsed acetate amendment, acetate-oxidizing iron and sulfate reducing bacteria, seasonal water table variation, and spatially-variable physical (hydraulic conductivity, porosity) and geochemical (reactive surface area) material properties. A particular challenge is the sensitivity of uranium mobility to pH, Eh, alkalinity, calcium, and reactive surface area, which are impacted directly by the terminal electron accepting process (TEAP) reactions and indirectly by subsidiary reactions involving the biologically-mediated reaction products. We use the eSTOMP subsurface simulator to exploit the large memory and high-performance of massively parallel computers needed to address the high spatial and temporal resolution, large number of reactive species and minerals, and detailed process models.

Our current work focuses on the mechanistic, predictive and quantitative understanding of the bacteria that catalyze bioreduction in the Rifle aquifer. Proteomics data from the 2008 field experiment provided an unprecedented opportunity to assess the capabilities of a genome-scale metabolic ("*in silico*") model of *Geobacter metallireducens,* putatively responsible for the reduction of Fe(III) and U(VI) (Lovley et al., 1991). The *in silico* model of physiological metabolic pathways that was embedded in the field-scale modeling framework is comprised of hundreds of intra-cellular and environmental exchange reactions. One advantage of this multiscale modeling approach is that the TEAP reaction stoichiometry and rate is now a function of the metabolic status of the microorganism, which is affected by acetate and nutrient transport, and biogeochemical conditions. The annotation of *in silico* model reactions to specific *G. metallireducens* proteins and the availability of groundwater proteomic analyses were instrumental to the assessment of model accuracy under evolving hydrologic and biogeochemical conditions. In this case, the largest predicted fluxes through *in silico* model reactions generally correspond to high abundances of proteins linked to those reactions (e.g., some central metabolic pathways). Model discrepancies with the proteomic data, such as the prediction of shifts associated with nitrogen limitation, identified pathways requiring further investigation and potential model refinement to better understand and more accurately predict metabolic processes that occur in the subsurface. The potential outcome of this approach is a predictive understanding of the interplay of key microbial species represented by multiple "*in silico*" models developed, in part, using gene networks identified from metagenomic datasets, and validated using global proteomics.