Microbiological-enhanced mixing across scales during *in-situ* bioreduction of metals and radionuclides at Department of Energy Sites

University-Led Research

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Bioreduction is being actively investigated as an effective strategy for subsurface remediation and long-term management of DOE sites contaminated by metals and radionuclides (i.e. U(VI)). These strategies require manipulation of the subsurface, usually through injection of chemicals (e.g., electron donor) which mix at varying scales with the contaminant to stimulate metal reducing bacteria. There is evidence from DOE field experiments suggesting that mixing limitations of substrates at all scales may affect biological growth and activity for U(VI) reduction.

Although current conceptual models hold that biomass growth and reduction activity is limited by physical mixing processes, a growing body of literature suggests that reaction could be enhanced by cell-to-cell interaction occurring over length scales extending tens to thousands of microns. Our project is investigating two potential mechanisms of enhanced electron transfer. The first is the formation of single- or multiple-species biofilms that transport electrons via direct electrical connection such as nanowires through biofilms to where the electron acceptor is available. The second is through diffusion of electron carriers from syntrophic bacteria to dissimilatory metal reducing bacteria (DMRB). The specific objectives of this work are i) to quantify the extent and rate that electrons are transported between microorganisms in physical mixing zones between an electron donor and electron acceptor (e.g. U(IV)), ii) to quantify the extent that biomass growth and reaction are enhanced by interspecies electron transport, and iii) to integrate mixing across scales (e.g., microscopic scale of electron transfer and macroscopic scale of diffusion) in an integrated numerical model to quantify these mechanisms on overall U(VI) reduction rates.

We are testing these hypotheses with five tasks that integrate microbiological experiments, unique micro-fluidics experiments, intermediate-scale flow cell experiments, and multi-scale numerical models. Continuous fed-batch reactors will be used to derive kinetic parameters for DMRB, and syntrophic/DMRB systems. They will also be used to develop an enrichment culture for elucidation of syntrophic relationships in a complex microbial community. Pore and continuum scale experiments using microfluidic and bench top flow cells will be used to evaluate the impact of cell-to-cell and microbial interactions on reaction enhancement in mixing-limited bioactive zones, and the mechanisms of this interaction. The microfluidic experiments will be used to develop and test a pore scale model that considers direct cell-to-cell interactions during U(VI) reduction. The pore scale model will be incorporated into a multi-scale hybrid model that combines pore scale modeling at the reaction interface with continuum scale modeling. We will validate the multi-scale model by comparison with continuum scale bench top flow cell experimental results, and then explore opportunities to extend this model to larger systems and eventually to field sites.

Work to date for this new project has investigated developing an appropriate system of syntrophs and metal reducers for use in follow-up experiments. Preliminary results indicate that *Anaeromyxobacter dehalogenans* has the capability to grow syntrophically with *Syntrophobacter fumaroxidans* using propionate as an electron donor and ferric citrate or ferrihydrite as an electron acceptor. Similarly, *Geobacter lovleyi* has grown with *S. fumaroxidans* using propionate and ferrihydrite only. In contrast, *G. sulfurreducens* has not been capable of syntrophic growth. Preparatory work needed for design and fabrication of microfluidic flow cells has been completed. Finally, we are starting to formulate the mathematical model for diffusion and reaction with a biofilm consisting of both syntrophs and metal reducers.