

## Final Report

Coupling in silico microbial models with reactive transport models to predict the fate of contaminants in the subsurface.

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This project successfully accomplished its goal of coupling genome-scale metabolic models with hydrological and geochemical models to predict the activity of subsurface microorganisms during uranium bioremediation. Furthermore, it was demonstrated how this modeling approach can be used to develop new strategies to optimize bioremediation. The approach of coupling genome-scale metabolic models with reactive transport modeling is now well enough established that it has been adopted by other DOE investigators studying uranium bioremediation. Furthermore, the basic principles developed during our studies will be applicable to much broader investigations of microbial activities, not only for other types of bioremediation, but microbial metabolism in diversity of environments. This approach has the potential to make an important contribution to predicting the impact of environmental perturbations on the cycling of carbon and other biogeochemical cycles. A detailed account of published accomplishments follows.

The first genome-scale, constraint-based model coupled to geochemical and hydrological models was developed. In order to accomplish this we first constructed a 'hybrid constraint-based model' in which Michaelis-Menten parameters for uptake of electron donor, acceptor, and key nutrients are used to describe the rate of supply of substrates to the cells and then the constraint-based model calculates the cell yield for those uptake rates. Initially acetate, Fe(III), and ammonium were evaluated as limiting nutrients. The physiology of subsurface *Geobacter* was modeled for 1000 possible combinations of environmental conditions during *in situ* uranium bioremediation, representing all possible combinations for ten discrete, environmentally relevant concentrations each for acetate (0-3 mM), Fe(III) (0-10 mmol/liter), and ammonium (0-1 mM). The hybrid model predicted no growth of *Geobacter* for 270 of the 1000 different potential environmentally relevant combinations of acetate, Fe(III), and ammonium availability that were modeled. It was predicted that under these conditions, the concentration of one or more substrates was too low to supply enough ATP for maintenance energy requirements. For the remaining 730 combinations of nutrient availability the biomass yield varied from 2.5 to

3.2 grams of cell dry weight per mole of acetate consumed. The same growth yield was possible for different types of nutrient limitation, depending upon the concentration of all three nutrients. These results indicate that growth yield can vary substantially based upon nutrient availability and that different physiological states can have similar biomass yields. This variable yield output of the hybrid model is in marked contrast to previously described microbial components for bioremediation in which growth yield is assumed to be constant. The hybrid model generally predicts much lower (and more realistic) growth than standard modeling approaches in which yields are based on the amount of energy theoretically possible from the reaction.

Another interesting output of the hybrid model, was the prediction of minimum thresholds for electron acceptors and electron donors. The prediction of a threshold for Fe(III) is in agreement with field results and laboratory incubations of subsurface sediments that have suggested that Fe(III) reduction stops and sulfate reduction begins before all of the readily reducible Fe(III) has been reduced. This is an important consideration not only for modeling the distribution of *Geobacter* species in the subsurface, but also the competition between Fe(III) reducers and sulfate reducers.

The hybrid model was coupled with the HYDROGEOCHEM reactive transport model. The growth and activity of *Geobacter* at the 1000 possible combinations of environmentally relevant concentrations of acetate, Fe(III), and ammonium served as a lookup-table. At each time step a call is made to the lookup-table to define the reaction fluxes of constituents and biomass yields. The data on uptake is then feed back to the reactive transport model as reaction rates for the current time step, and the reactive transport model is stepped forward one time interval, after which the process is repeated. This approach alleviates the need for the more computationally intensive strategy of solving the hybrid model for each cell of the plot grid at each time interval. The coupled microbial hybrid-HYDROGEOCHEM model accurately described the acetate and U(VI) profiles that were observed during the 2002 in situ uranium bioremediation field experiment at the DOE study site in Rifle, CO. This was accomplished without the parameter fitting exercise that was required in previous attempts to model this field experiment. A manuscript describing these results was published in *Microbial Biotechnology*.

Next the modeling approach was expanded to examine interactions between different microbial species. For example, in the 2007 field study at the Rifle, CO study site it was found that there was substantial heterogeneity in the distribution of ammonium. Prior to the addition of acetate to initiate stimulated bioremediation, *Rhodoferrax* species were more abundant than *Geobacter* species at the site with high ammonium, whereas *Geobacter* species predominated where ammonium availability was low. When acetate was added to the subsurface *Geobacter* species become the predominant dissimilatory metal-reducing microorganisms, regardless of ammonium availability. Modeling of the interaction between *Geobacter* and *Rhodoferrax* revealed that *Rhodoferrax* is favored over *Geobacter* when the flux of acetate is low and ammonium is available. This is because *Rhodoferrax* has a higher growth yield than *Geobacter*. However, *Rhodoferrax* cannot fix nitrogen, and thus *Geobacter*, which can fix nitrogen, can compete with *Rhodoferrax* in

environments with low electron donor flux if ammonium is limiting. Although *Geobacter* has a lower yield than *Rhodoferrax*, it has a much higher growth rate. Therefore, when acetate is added to the subsurface *Geobacter* readily outcompetes *Rhodoferrax* and becomes predominant. This modeling has provided important insights into why *Geobacter* species are consistently abundant constituents of the subsurface community when electron donors are added to the subsurface. A paper summarizing the modeling results was published in *ISME Journal* and a paper summarizing the field experiments that were used in development and verification of the modeling was published in *Environmental Science and Technology*.

The mathematical representation of the U(VI) reduction in the subsurface was significantly improved by including the concept that *Geobacter* species can continue respiration when they are not in contact with Fe(III) oxides by accumulating electrons in their abundant c-type cytochromes. In order to accomplish this a multi-scale HydroBioGeoChem model that integrates electron capacitance and the genome-scale metabolic model with hydrologic transport, heat transfer, and biogeochemical transport was developed for a flow-through column consisting of 30 elements and 62 nodes. The look-up table, consisting of 720 growth modes under 1000 different environmentally relevant combinations of acetate, Fe(III) and ammonium, was generated by ILOG CPLEX optimization engine built in Tomlab for Matlab and was seamlessly incorporated with the HydroBioGeoChem model through 4 point Newton interpolation formula. The cell-scale electron capacitance mechanism was coupled to HydroBioGeoChem through ordinary differential equations and a novel algorithm for calculating the stoichiometric coefficients. The model is capable of calculating species concentrations over a 2 dimensional grid at any desired time. Whereas the model that did not take electron capacitance into account was unable to predict effective U(VI) removal at low initial cell concentrations, the model with electron loading and unloading successfully describe the significant U(VI) reduction associated with acetate amendment. These results demonstrate that electron capacitance is not only important for energy generation when cells are temporarily out of contact with Fe(III) oxides, but also critical for U(VI) reduction in sediments. This is an important new insight into the mechanisms for in situ uranium bioremediation. A manuscript summarizing these results was published in the *Journal of Contaminant Hydrology*.

Interactions between *Geobacter* species and sulfate reducers were modeled in order to better understand the succession of microbial processes during in situ uranium bioremediation and to aid in designing strategies for extending the phase in which *Geobacter* species predominate and uranium is effectively removed from the groundwater. Batch simulations of community dynamics were used to examine the cause of the late onset of sulfate-reduction using different percentages of starting inoculants of *Geobacter* and sulfate reducers. These simulations showed that no matter what the starting conditions, *Geobacter* species were able to dominate the early phase of the experiment with very little change in timing of the onset of sulfate reduction. The field-scale simulation was able to accurately predict the field data, and suggested that the succession that is observed in the field is the result of the much faster growth rate of *Geobacter* species rather than competition for acetate. This model was used to define

when addition of Fe(III) to the subsurface would be most effective in continued promotion of the activity of *Geobacter* species. These model predictions were further evaluated experimentally. When active Fe(III)-reducing sediments were mixed with fully reduced, active sulfate-reducing sediments both sulfate-reduction and Fe(III)/U(VI)-reduction took place simultaneously. The addition of Fe(III) oxide to active sulfate-reducing sediments resulted in increase in the numbers of *Geobacter* species, but sulfate reduction continued. These results suggest that in high sulfate systems, acetate addition for U(VI) bioremediation is not a sustainable process due to the eventual growth of sulfate reducers that are likely to cause biofouling and clogging of the aquifer. Two manuscripts summarizing different aspects of these results were published, one in *Biogeosciences* and one in *Biotechnology and Bioengineering*.

Over time the coupling of the genome-scale modeling and reactive transport modeling was improved. A method was developed in which the genome-scale model is called directly by the reactive transport simulator during the field-scale simulation process. To avoid computational redundancy, output from the constraint-based model runs are stored in a database indexed to the associated combination of constraining concentrations. During subsequent time steps, the database is interrogated to determine whether a “similar” (within a certain distance in N-dimensional space) run was made previously and if so those results are used; otherwise, a new constraint-based optimization is performed. This approach was tested and sensitivity of model results to the selected distance tolerance was evaluated. These results were published in a paper in *Journal of Contaminant Hydrology*.

A study evaluating aspects of modeling the regulation of metabolism was published in *BMC Research Notes* and study on strategies for modeling anaerobic respiration was published in *Molecular Systems Biology*. A review article summarizing the concept of applying genome-scale metabolic modeling to the study of subsurface microbiology was published in *Nature Reviews Microbiology*.

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