Workshop on Modeling Bioremediation of Mixed Waste Sites

January – February 2008

CO-CHAIRS: JACK PARKER, Univ. of Tennessee; TONY PALUMBO, Oak Ridge National Lab

PRESENTERS: FRANK CHAPELLE, US Geological Survey; YILIN FANG, Pacific Northwest Lab; TERRY HAZEN, Lawrence Berkeley Lab; JACK ISTOK, Oregon State Univ.; PETER KITANIDIS, Stanford Univ.; JIAN LUO, Georgia Tech; TIM SCHEIBE, Pacific Northwest Lab; MARK WIDDOWSON, Virginia Tech; STEVE YABUSAKI, Pacific Northwest Lab

PARTICIPANTS: KRISHNA MAHADEVAN, Univ. of Toronto; DAN BOND, Univ. of Minnesota; SCOTT BROOKS, Oak Ridge National Lab; BILL BURGOS, Pennsylvania State Univ.; ERIC RODEN, Univ. of Wisconsin; CHRIS SCHADT, Oak Ridge National Lab; PAT SOBECKY, Georgia Tech; FAN ZHANG, Oak Ridge National Lab

A web-based workshop sponsored by the US Department of Energy Environmental Remediation Sciences Program (DOE-ERSP) was organized to assess the current state-of-the-science and knowledge gaps associated with modeling microbially-mediated remediation of mixed wastes, and options to expedite development of practical field-scale models for evaluating remediation performance at sites contaminated with radionuclides, metals and other contaminants.

DOE is responsible for managing legacy wastes associated with Cold War era nuclear weapons development and production, including an estimated 6.2 million cubic meters of buried waste containing 50 million curies of radioactivity and numerous other contaminants. ERSP directs research efforts aimed at improving the ability of models to simulate coupled field-scale hydrologic, geochemical and biological processes to better manage mixed waste sites at DOE facilities. Microbially-mediated reactions are arguably the most complex and uncertain component of current simulation models.

Individuals from academia and DOE laboratories with primary expertise in modeling or microbiology and experience involving groundwater bioremediation were invited to participate in the workshop. Two-hour meetings were held weekly for a 5-week period from 23 January to 20 February 2008 using Live Meeting bridged to a teleconference to provide linked audio and video communication. Meeting time was about equally divided between presentations and discussion. The system worked very effectively, even with a 12-hour time zone spread with one of the speakers (our thanks to Peter Kitanidis for getting up in the wee hours of the morning during a sabbatical in Singapore!) Presentations and reference materials were archived on a workshop web site to allow participants to review materials later or catch up if they missed a session.

State-of-the-Science

Over the last 20 years, advances in computer hardware and software have made field-scale simulations of hydrologic processes coupled with complex chemical and biological reaction networks computationally feasible. Computer codes are now available that are capable of modeling three-dimensional transient multiphase fluid flow under nonisothermal conditions and transport of multiple chemical species subject to coupled kinetically or equilibrium-controlled reactions associated with various physical, chemical and biological processes (e.g., Yeh et al., 2004; Fang et al., 2003). While the level of complexity in such models is theoretically unlimited, practical limits inevitably occur for actual model applications. Computational feasibility or cost

may impose constraints, although these factors have steadily diminished with technological advances. More often, data limitations, model sensitivity, and/or accuracy requirements combine to constrain models. For example, Liu et al. (2002) found a simple first-order model for iron-reduction kinetics to be more reliable than a more complex Monod-type formulation, because the larger number of parameters in the latter model could not be determined with precision from the available data. Furthermore, if predictions of interest are insensitive to certain processes or variables, then it would clearly be advisable to simplify the model accordingly. Significant advances have been made in methods for calibrating models to available data and determining optimal conceptual model and data requirements for a given problem application (Hill, 1998; Vrugt et al., 2003; Vrugt and Robinson, 2007; Tonkin et al. 2007).

Models for aerobic and anaerobic biodecay of organic chemicals are used routinely now to evaluate remediation alternatives and to assess regulatory compliance (e.g., Aziz and Newell, 2002; Widdowson, 2002). Although these systems are complex in terms of biochemical processes, microbial populations and interactions with numerous environmental variables, over timeframes relevant to remediation projects (i.e., months to years), sensitivities to many of these factors recede allowing models to be simplified and data requirements reduced (Aziz and Newell, 2002). In particular, it is well established that microbial community dynamics and biodecay rates for organic contaminants are largely controlled by electron donor, electron acceptor and/or nutrient availability, and that long-term bioremediation performance is generally insensitive to initial microbial population (the presence of *Dehalococcoides* needed for anaerobic biodecay of chlorinated solvents is an exception). Thus, biostimulation of indigenous microorganisms is generally preferred over bioaugmentation.

Bioremediation of metals and radionuclides is a less mature technology. While organic bioremediation involves irreversible transformations to innocuous products (if the reactions proceed far enough), bioremediation of metals and radionuclides involves reactions that reduce contaminant mobility by potentially reversible precipitation, sorption or complexation reactions that can involve rather complex interactions between geochemical and microbial processes. The kinetics of remobilization reactions is thus an important concern. Models for microbial growth and reaction kinetics have been described for Fe, Mn and various trace metals in pure and mixed cultures (Truex et al., 1997; Spear et al., 1999; Liu et al., 2002; Bandtra, 2005; Lim et al., 2007). Several field-scale applications of coupled flow, transport, and biogeochemical reaction models that describe microbial growth with Monod kinetic formulations have been reported recently (Scheibe et al., 2006; Yabusaki et al., 2007; Luo et al., 2008). However, sensitivity analyses suggest that microbial reduction of uranium is primarily limited by the availability of electron donor species (unpublished results, Kitanidis and Luo), which is consistent with the extensive literature on bioremediation of organic contaminants.

Istok et al. (2008) have proposed a strictly thermodynamic approach for modeling microbially mediated reactions. Microbial groups are defined on the basis of common metabolic capability in terms of electron donor and electron acceptor species utilized. Chemical equations are formulated for each group and growth equations derived from bioenergetic principles (Rittman and McCarty, 2001). The free energy yield for each group is computed from the reaction stoichiometry and a parameter (calibrated to experimental data) that partitions energy obtained from electron donor oxidation between cell maintenance and growth. Reaction rates are assumed to be fast relative to residence times (i.e., reaction rates are transport-limited). The method was successfully applied to various lab and field data sets. Because the approach involves many

fewer parameters with a narrower feasible range than conventional kinetic models, the thermodynamic approach should facilitate practical model calibration and allow a more comprehensive microbial community to be modeled.

Significant advances have been made in proteomic and genomic characterization of microbial populations and the identification of gene groups associated with various functional processes using molecular biological techniques.

The discovery of new microbial metabolic pathways can transform the way we understand microbial contributions to biogeochemical processes. For example, recent discoveries of new pathways important for nitrogen and carbon cycling have added to our understanding of these processes (Arrigo 2005, Capone and Knapp 2007; Francis et al 2007). Molecular methods are now available to enable clone library construction and phylogenetic analysis (Yan et al., 2003; Palumbo et al., 2004; Fields et al., 2005) and tracking of functional gene targets using quantitative polymerase chain reaction (QPCR) and terminal restriction fragment length polymorphism (TRFLP) (Scala and Kerkhof, 1999) and functional gene microarrays (Schadt et al., 2005; Wu et al. 2006). These and other methods have led to a burgeoning database of molecular-level information on microbial populations.

These techniques have proven useful for field-scale process monitoring and model refinement at the uranium bioremediation site in Rifle, Colorado (Yabusaki et al., 2008). Research is in progress to investigate quantitative relationships between genome- and molecular-scale microbiological data and reaction kinetics. For example, genome-scale metabolic models of *Geobacter* species (metal-reducing subsurface bacteria) have been used to refine thermodynamically-based estimates of growth yields (Sayyar et al., 2008), which could improve parameterization of kinetic reaction network models and the thermodynamically-based modeling approach described above. Furthermore, an effort is underway to couple constraint-based genome-scale metabolic models with reactive transport models (e.g., in lieu of Monod-based rate formulations with fixed yields) to more accurately represent changes in microbial functions in response to environmental conditions. Preliminary results of this approach at the Rifle site have been promising (Fang et al., 2008; Garg et al. 2008).

Research Issues

An overarching research priority noted by workshop participants is the identification of rate-limiting processes for microbial growth and microbially mediated chemical transformations and the formulation of relevant rate equations. It seems probable that availabilities of electron donor, electron acceptor, and key nutrient species are primary limiting factors in many circumstances, although inhibitory species, microbial competition, predation, transient metabolic states and other factors may be dominant in certain cases.

The equilibrium reaction path model of Istok et al. (2008) assumes microbial reaction rates are limited by the transport of reactant species, resulting in significant reductions in the number of uncertain model parameters. The thermodynamic framework also ensures that thermodynamically unfavorable reactions do not proceed, while conventional Monod models may allow them to occur. However, further work is warranted to determine if additional factors significantly limit reaction rates in certain circumstances and if so, how the rate expressions can be formulated. For example, at large space scales, nonequilibrium distributions of reactants, products and biomass within heterogeneous aquifers may control reaction rates (which might be modeled as a mass transfer problem).

More controlled experiments are needed that are specifically designed to provide adequate data to test and refine reaction models. All microbial models involve the introduction of functional microbial "groups" based primarily on redox couples. Methods are needed to experimentally determine modeled microbial groups from genomics data to verify and refine model predictions of microbial community dynamics. This will require the identification of microbial groups whose metabolism can be measured by molecular biological techniques. Growth yields and reaction stoichiometries should be compiled for relevant functional microbial groups in the context of biogeochemical conditions.

Because problems of practical interest occur in very heterogeneous subsurface conditions at the scale of kilometers, while measurements are obtained at much smaller scales, scaling is another priority issue with broad implications for the formulation of *all* modeled processes, including reaction kinetics. Spatial heterogeneity in material properties and microbial populations and temporal variations in boundary conditions always exist at scales less than those explicitly represented by a model. Sub-grid variance generally diminishes as resolution increases. Upscaling and multiscale modeling methods can be useful for elucidating scale effects and for developing better model formulations. However, data limitations will constrain the representation of small-scale variability (explicitly or via multiscale methods) in practical model applications. Model scale not only affects parameter values but can impact the apparent form of constitutive relations. For example, cellular processes may limit growth at the pore scale, but at larger scales mass transfer between mobile and immobile regions or high and low population regions, which follow different rate functions, may control microbial rates.

The issue of "optimal" model complexity was discussed, both from the standpoint of time/space scale resolution and from the perspective of details of processes to be considered or disregarded. Optimality always implies tradeoffs. Although intrinsic error may diminish with greater resolution and conceptual detail, more data will be needed to control prediction error associated with uncertain model parameters. Thus, optimal complexity represents a tradeoff between the benefit of reduced prediction uncertainty and the cost of obtaining more or better calibration data (Figure 1). Uncertainty analysis can serve a useful role as a programmatic border collie to nip at the heels of models that stray too far from the desired path.

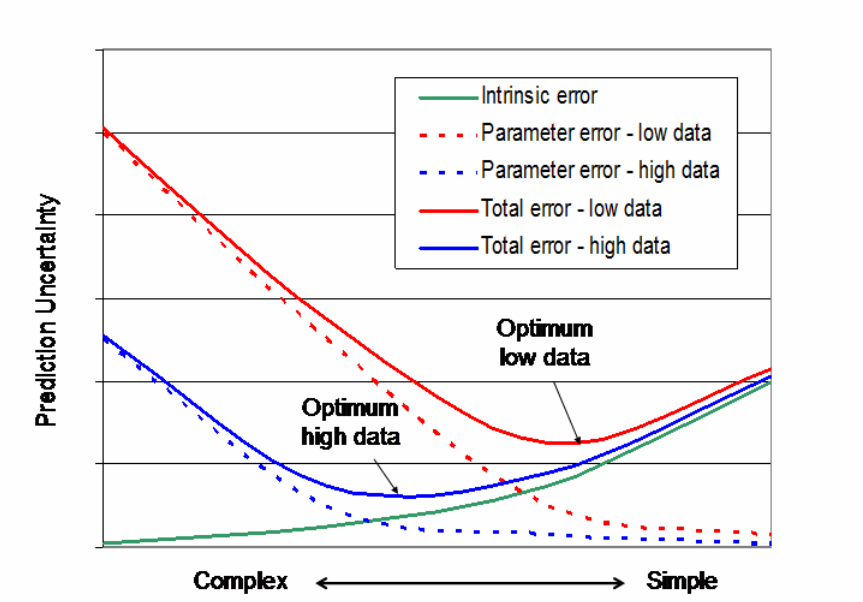

Figure 1. Hypothetical prediction uncertainty versus model complexity

The foregoing does not imply that more complex or highly resolved models than may be warranted for specific field-scale applications do not serve an important role. Such models are invaluable for performing numerical experiments that may identify the limitations of simpler models, and they can be used to develop or test improved upscaled constitutive models subject to a more rigorous range of conditions, and to test the integration of advanced characterization techniques, which may ultimately lead to improved modeling approaches. Furthermore, the process of determining optimal practical model complexity must necessarily consider both simpler and more complex models than may be optimal. However, while striving to improve simulation models, practical limits on model complexity imposed by tradeoffs between prediction uncertainty and data availability should not be forgotten.

Microbiologists and modelers tend to look at the same system quite differently. This workshop served as a step towards narrowing the communication gap and turning divergent perspectives towards a single focal point. Given a defined functional program objective, models can serve a useful role in setting research priorities. For example, if a goal is to improve predictions of field-scale remediation performance and if simulated performance is found to be sensitive to uncertainty in process B and insensitive to that of process A, then it will be beneficial to focus subsequent efforts on refining our understanding of process B. A collaborative protocol of iterative refinement driven by model-derived priorities (Figure 2) will advance both basic scientific understanding and practical applications of mixed waste site bioremediation more rapidly. Programmatic efforts to improve integration between modeling and experimental studies are likely to pay significant dividends. Efforts to resolve basic science questions may also identify gaps in understanding that ultimately lead to improvements in future generations of models.

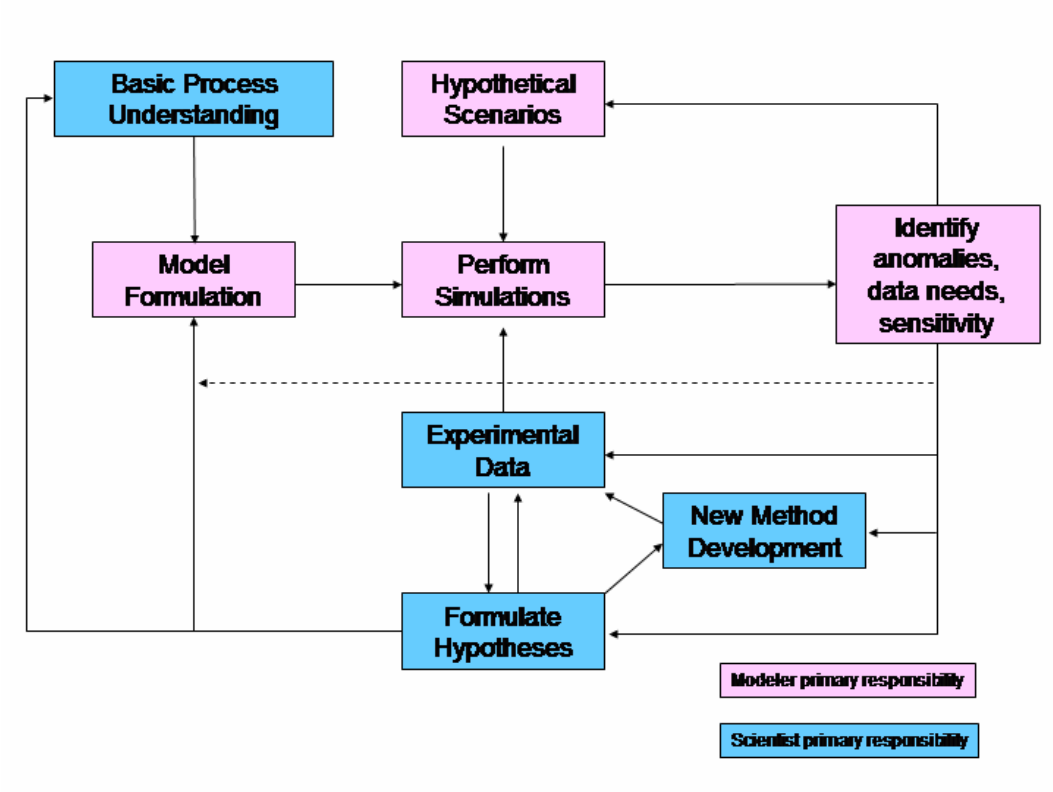

Figure 2. Collaborative model development flowchart

The following research needs were identified to advance the predictive capability of subsurface reactive transport models and their practical application at field sites:

- Emphasize collaborative model development and testing by integrated teams of specialists undertaking iterative refinement, experimentation and hypothesis-testing driven by model-derived priorities.
- Utilize state-of-the-art models to determine model limitations and data needs and perform controlled experiments to test improved model formulations.
- Develop thermodynamic biogeochemical reaction networks that address both abiotic and biotic reactions with growth yields and reaction stoichiometries for functional microbial groups.
- Determine relationships between biogeochemical conditions, metabolic status, and stoichiometry and kinetics of biologically-mediated reactions.
- Develop methods to identify functional microbial groups from genomics data to verify and refine microbial community dynamic models.
- Identify processes that limit rates of microbial growth and microbially-mediated reactions at the field scale (e.g., reaction kinetics, reactant distributions) and formulate relevant mathematical models.

- Utilize upscaling and multiscale modeling methods to elucidate scale effects, and develop improved constitutive model formulations that can be realistically implemented in field-scale models.
- Develop improved models, calibration methods and error analysis techniques that can utilize multiscale characterization data (including geophysical measurements, genomics-based microbial community structure data, etc.) to improve accuracy and reduce uncertainty of field-scale model predictions.
- Evaluate tradeoffs between model scale and complexity and data available for model calibration on the one hand and forward prediction accuracy and uncertainty on the other hand.
- Take advantage of advanced computational capabilities to improve understanding of complex coupled systems, and to develop more cost-effective design of remediation, characterization and long-term monitoring systems.

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Author Information

JACK PARKER, University of Tennessee (co-chair); TONY PALUMBO, Oak Ridge National Laboratory (co-chair); FRANK CHAPELLE, US Geological Survey (presenter); YILIN FANG, Pacific Northwest Laboratory (presenter); TERRY HAZEN, Lawrence Berkeley Laboratory (presenter); JACK ISTOK, Oregon State University (presenter); PETER KITANIDIS, Stanford University (presenter); JIAN LUO, Georgia Tech (presenter); TIM SCHEIBE, Pacific Northwest Laboratory (presenter); MARK WIDDOWSON, Virginia Tech (presenter); STEVE YABUSAKI,

Pacific Northwest Laboratory (presenter); KRISHNA MAHADEVAN, University of Toronto; DAN BOND, University of Minnesota; SCOTT BROOKS, Oak Ridge National Laboratory; BILL BURGOS, Pennsylvania State University; ERIC RODEN, University of Wisconsin; CHRIS SCHADT, Oak Ridge National Laboratory; PAT SOBECKY, Georgia Tech; FAN ZHANG, Oak Ridge National Laboratory

Corresponding author: Jack Parker, University of Tennessee, jparker@utk.edu