NABIR Subcommittee Report on the Bacterial Transport Element
Meeting held, Oct. 10-11, 2000
AGU Building, Washington D.C.

The BERAC subcommittee for NABIR was asked to address some general and specific issues and questions on all elements of NABIR, starting with the bacterial transport element. The following is the subcommittee's report on the bacterial transport element in response to the questions the committee was asked to address for this program element. The report is organized to follow the topics and questions asked of the subcommittee.

I. RELEVANCE, IMPACT AND UNIQUENESS

1. <u>Is the microbial transport research an appropriate part of NABIR's bioremediation research strategy</u>? The microbial transport research is mainly relevant to the select cases where specialized organisms must be introduced. One example of a potential remediation scenario where bioaugmentation might be needed is for the bioremediation of complex mixed waste (e.g. plutonium/carbon tetrachloride waste at Hanford). Results from the bacterial transport research are also applicable to fate issues broadly associated with bioremediation activities of different types. This research is not very relevant for biostimulation approaches for metal or radionuclide remediation, except in defining the transport of organisms post-stimulation. The current program is heavily focused on geology and hydrology with the microbial ecology, especially the key activities, under emphasized compared to needs for solving DOE problems.

2. <u>Is the Oyster site research focused on gaps in scientific understanding</u>? Yes it is focused on those gaps and is based on good understanding of the current state of science. Initial experiments have focused on resolution of chemical/physical, and hydrogeologic issues, emphasizing heterogeneity. The group is now poised to address microbiologic issues that the panel thought could be quite important. The knowledge developed at the Oyster site provides the necessary background to allow defensible testing of field-scale microbiological hypotheses.

3. <u>Are the goals and priorities of the ongoing and planned research clear and achievable</u>? The goal, "to develop the understanding of microbial transport processes in subsurface environment" is clear and worthy from a scientific prospective. The stated goals with respect to bioremediation/acceleration are not clear.

4. <u>Is the research consistent with the overall NABIR goals</u>? The linkage of the goals of the bacterial transport program to the NABIR program is unclear. The consistency of the bacterial transport research with the NABIR program could be enhanced by focusing future efforts at a contaminated site(s). Sites in addition to the FRC, which have unique characteristics, should be considered. Effort should be made to fully transition research

conducted under this program within the next three years to DOE contaminated site(s) and/or other sites with demonstratable relevance to DOE sites in terms of such characteristics as contaminants, hydrogeology, or microbial ecology. Plans must be developed immediately to accomplish this goal.

5. Is this research complementary to research funded by other agencies? Yes, this research is filling a niche not being filled by other agencies. It is complementary to work funded by USGS (virus transport), NIEHS (transport of pathogens), EPA (non-point pollution and water sheds), and USDA (animal waste).

6. What are the unique attributes of this research compared to efforts funded by other agencies? The unique attributes of this work include the focused interdisciplinary characteristics of the research team, the comprehensive scientific scope of the effort, the synergy among the investigators, and the link between modeling and experimentation.

7. Will the current/planned research help EM fulfill its mission (stabilizing or remediating legacy waste)? This research will help EM fulfill its mission if bioaugmentation is required. However the need for bioaugmentation and the conditions under which it might be useful are not well established. Supporting technologies developed by this research (e.g. microbial tracking technologies, geophysical characterization technologies, and down-hole characterization techniques) may have broad applicability to EM sites. The microbial transport/modeling capability could also have applicability to general bioremediation activities on DOE lands. The microbial transport research could have enhanced impact on EM activities if the research targeted defensible remediation scenarios (e.g., the interception of uranium or technecium plumes of certain dimensions and depths).

8. Would the current research lead to the ability to predict which DOE sites are amenable to bioaugmentation? The research will identify factors and processes controlling bacterial transport at the Oyster site. Descriptive field scale models of the process will be constructed. Both of these activities would yield qualitative insights that may be applied to other sites. The stated question cannot be fully addressed until the nature of the sites where bioaugmentation is necessary to transform or stabilize in-ground contaminants is defined.

II. CONNECTIVITY TO OTHER ELEMENTS OF NABIR PROGRAM

Researchers within the transport element are well integrated, reflecting strong DOE leadership and a well-organized research plan. It is desirable that this integration continue since multiple processes are involved in bacterial transport that must be quantitatively related, and in turn related to the in situ activities of the microbiota. While the research is well integrated within the element, it is not well integrated with the larger NABIR program. Potential research opportunities, in bacterial transport and related studies, at the Oyster site provide the strongest motivation for research integration with other NABIR elements.

We encourage active and effective integration across all elements of the NABIR program. Some investigators have taken the initiative to interact with researchers in other program elements and FRC investigators, which is to be commended. We strongly recommend that DOE foster formal and continuing interactions between all NABIR program elements. The Oyster site provides excellent opportunities for collaborative research with other elements. DOE should capitalize on these opportunities by supporting research that takes advantage of disciplines and strengths within the other program elements.

A range of mechanisms can be used to encourage collaboration with other elements. A few low-cost examples are: holding roundtable meetings at the annual PI meeting; early implementation of the Oyster Web site with links to the NABIR Homepage; and explicitly incorporating collaboration as a criterion into the project selection process. Any new RFAs should encourage collaborations across the NABIR program elements to address high-priority research questions.

The benefits of working at the Oyster site are: the high degree of hydrogeologic characterization that has been performed; the infrastructure that is present; the presence of different redox zones for conducting experiments; and the good interactions that have been established with the property owner and local community. Further, working at an uncontaminated, shallow groundwater site lowers the cost the experimentation. These attributes are amenable to sharing of samples and providing access to other researchers. Surface outcrops of the subsurface strata exist to aid in the characterization of the subsurface geology.

Some constraints of working at this site currently are the inability to inject nonindigenous microorganisms; possible limits on injection of substrates, e.g. electron and acceptors; that the indigenous microorganisms may not be representative of those at a contaminated site; and there may not be natural chemical analogs for metals and radionuclides relevant to DOE sites. These issues need to be addressed if the scope for this work is to extend beyond single organism transport. Transport of organisms, with properties useful to DOE site remediation, e.g. *Geobacter*, should be seriously considered. Relevance to DOE sites must be addressed in new proposals written for the Oyster site research. The research results, tools and methods should be transitioned to other DOE sites within the Oyster site constraints.

Based on the investment to date and the potential for additional high-quality, DOE-relevant investigation, research should continue at the Oyster site, transitioning to a contaminated site within three years. The Oyster site represents a hydrogeologic regime that is complementary to the FRC and extends NABIR research to a greater diversity to DOE site matricies. However, research on microbial transport should be extended to include understanding the role of microbial ecology in governing the fate of microbes in the subsurface.

III. TECHNICAL QUALITY

1. <u>Bacterial labeling and tracking technologies</u>. The bacterial labeling and tracking technologies represent one of the strong points of this program. There is good appreciation

of the interpretational limits and costs associated with the various procedures. This program has been particularly effective in bringing the multiple technologies to bear in a coordinated fashion and allowing the procedures to be cross-referenced. An additional strength of the labeling and tracking technologies is their transferability to other microorganisms and sites that are of interest to DOE. However, the subcommittee recognizes that the mere presence of microorganisms as indicated by the tracking methodologies is not synonymous with the metabolic activity of the inoculants. The panel encourages creative mechanisms to marry assessments of microbial activity with the existing tracking tools.

2. Issues of Physical and Chemical Heterogeneity. A second notable aspect of the research program is the development of novel methods to characterize subsurface heterogeneity. The combination of hydrologic and geophysical data appears to provide estimates that are consistent with the observed behavior of the tracer plume. Information about chemical heterogeneity is less clearly defined, which is likely due to the complexity in identifying such variability in a physically heterogeneous environment.

   The parameter estimation methods employed at the Oyster site are likely to define the physical heterogeneity at scales necessary to improve predictions of tracer and bacterial transport relative to conventional methods. Small-scale chemical heterogeneity is likely to influence transport at the field scale, however macroscale parameters are likely able to adequately describe the processes important to interpret the field data.

   The subcommittee believes that more effort is needed in processing the available data to characterize chemical heterogeneity. In addition, more effort is needed to understand the influence of aquifer properties on microbial processes such as attachment and detachment given the immense quantity of available data. The planned examination of the incremental value of data provides one mechanism to transfer knowledge from this characterization effort to future investigations at contaminated sites where collection of such data is generally much more costly.

3. Hydrologic and bacterial transport models. The modeling approaches developed through the Oyster work show promise for addressing microbial transport problems at this site. At this point it appears that the post modeling that incorporates the characterization information has not been incorporated. The modeling tools that are being used at the site should be able to interpret the collected data. Models should continue to remain process-oriented to facilitate future transfer of the approaches and results to other field sites. Transition to DOE legacy sites requires incorporation of additional processes into the models such as growth and decay in response to addition of an electron donor or other nutrients.

4. Isolation and characterization strategy. The Oyster investigators are to be complimented for their efforts to isolate and characterize bacterial strains that fit within a constraining regulatory framework. For instance, the selection of inoculants that are sensitive to as many as eight antibiotics is hardly scientifically defensible, but rather a product of concerns dictated by the Nature Conservancy. The subcommittee recognizes that these constraints are

hard to anticipate and understands that the investigators have succeeded in building a trust that may ultimately facilitate additional experimentation. The subcommittee also recognizes that the regulatory climate can substantially vary among states and even among sites in a given state. Information about the process of working in concert with local regulators, property owners, community members, etc. may be transferable, at least to some extent, to other sites.

The underlying strategy of the investigators is clearly to select and enrich for microorganisms that show relatively little ability to adhere to the subsurface matrix. The transport of these inoculants is then evaluated relative to water soluble tracers and the success of such endeavors is interpreted relative to the physical and chemical heterogeneities of the subsurface. While this strategy is appropriate and even scientifically defensible, nonadherent cells are often the exception to most subsurface bacterial life forms. As such, the strategy of attempting to disperse cells throughout a contaminant plume may not always be a desirable or even feasible endeavor. Approaches for contaminant remediation can also be married to other inoculation approaches (i.e. biobarriers, etc.). Consequently, the subcommittee feels that at least a some effort to understand the mechanisms of cell transport by adherent organisms (i.e. growth, chemotaxis, etc.) is also warranted. Fe reducers are notoriously adherent and earlier studies have indicated that most of the organisms in natural populations will migrate less than a meter from the point of injection.

5. <u>Design of infrastructure of the field flow cells</u>. The field design for the site incorporated several innovative approaches that further advanced with the design of the SOFA site. The iterative process between modeling and design provided a field grid that appears to have captured the important aspects of transport during the experiment. As a result of the well designed infrastructure, further research at the site should be able to answer fundamental questions about microbial processes. A weakness is the difficulty in studies of anaerobic processes likely to be important in DOE contaminated sites.

6. <u>Current research agenda</u>. The Oyster group has clearly incorporated an appropriate range of disciplines in the research program. The program is now well-positioned to carry out scientifically defensible controlled field experiments that build on the enormous characterization and modeling efforts to date. The panel recommends that the research group bring the available data together in a mode that allows for an improved understanding of fate, transport, and activity of microbes in heterogeneous environments.

IV. SUMMARY AND RECOMMENDATIONS.

It is obvious that the microbial transport element is well-integrated but has developed independent of the broader NABIR program and DOE sites needs. While the research is first rate and on track to provide definitive information on three dimensional microbial transport in sandy sediments, <u>it is time to converge this</u> element with the NABIR program goals and DOE site needs. In view of this need for convergence, we have the following recommendations:

1. We believe that the major scientific information for understanding microbial transport in aerobic sandy sites can be realized within 3 years. Hence, we recommend no further field campaigns at Oyster site beyond approximately 3 years from now.

2. That planning begin immediately for transitioning information and experimentation on transport and bioaugmentation relevant to DOE contaminated sites, including the FRC, and that a research plan (diagram) be written this year for this integration. This plan should include exploiting the excellent science and scientists from the Oyster project to focus on microbial ecology manipulations relevant to metal remediation.

3. That the element of transport of non-biostimulated cells, alone, is too narrow and should be expanded into the original concepts envisioned by acceleration. An increased portion of the effort should focus on improving our understanding of the mechanisms of cell transport by adherent organisms (i.e. growth, chemotaxis, etc.) due to the critical role these organisms will have in most biostimulation/bioaugmentation remediation efforts.

4. That the remaining work at the Oyster site focus on microbial fate, transport and activity so that the unique microbial information can be fully interpreted in the context of the site characterization, chemical and physical information. In addition we recommend that amendments, such as the addition of electron donor, be a treatment included so that key parameters of growth and activity can be evaluated at the field scale.

5. Since the general evidence is that Fe and metal reducing bacteria are widely distributed in nature, and hence the need for their bioaugmentation is not expected in most cases, the forthcoming transport experiments should emphasize aspects that are applicable beyond the focus on bioaugmentation of metal reducers. An example is the need to examine the transport properties of metal reducing microbes after biostimulation.

6. That the forthcoming RFP recognizes the recommendations under the above points.

Subcommittee members and guest experts present at the meeting:

Chester Miller	David Hyndman
John Zachara	Amy Wolfe
Derek Lovley	Cathy Vogel
Joseph Suflita	Raymond Wildung
Lew Semprini	David White
	James Tiedje

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