Report of NABIR Subcommittee of BERAC
May 7-8, 2002, Belmont House, Baltimore, MD

Biotransformation and Biogeochemistry Elements

Introduction. The NABIR Subcommittee addressed the seven questions below regarding program planning for the Biotransformation (BT) and Biogeochemistry (BG) elements. These are large program elements in the NABIR portfolio and involve many investigators. We find this emphasis to be appropriate because these elements address the core science needs underlying DOE's metal and radionuclide remediation problems. We were particularly impressed by the quality of the science presented to the Subcommittee. This research is already making important contributions to knowledge in the exciting new field of geomicrobiology and well as in geochemistry and microbiology.

1. Does the current portfolio of projects in these elements support the program and science element goals and objectives as articulated in the NABIR strategic plan?

The goals as articulated in the NABIR strategic plan are:

> BT Goal: To understand the mechanisms of microbially mediated transformation of metals and radionuclides in subsurface environments leading to immobilization and long-term stability *in situ.*
>
> BG Goal: To understand fundamental biogeochemical reactions leading to long-term immobilization of metal and radionuclide contaminants in the subsurface.

After considering the portfolio of projects, presentations, and results presented at annual PI meetings, the committee reached the conclusion that, yes, the portfolio of projects are meeting these goals.

Sharpening the focus of the NABIR program (i.e., research on *immobilization* of contaminants; *bio*remediation, and the elements *Cr, Hg, U, Tc, and Pu*;) has greatly facilitated the creation of a portfolio to meet program element goals. Furthermore, the well-focused scope enables assessments of the success of the portfolio in meeting programmatic goals.

Both program elements have two complementary targets: (a) high-quality science in a 3-year time frame and, (b) in a 10-year time frame, integration of coupled processes, testing in the field, and numerical models for assessing long-term stability. The committee believes that the program elements are meeting the first, 3-year target. However, to meet the ten-year target, steps will have to be taken soon to begin to address *field* and associated *modeling* issues in greater depth.

According to the NABIR Strategic Plan, the goals and targets of the BT and BG program elements are to develop a *science-based understanding* for long-term immobilization of metals and radionuclides. Also, the Strategic Plan states that the primary customer for

NABIR deliverables, with responsibility for *implementating appropriate technologies in the field*, will be DOE-EM, particularly the EM Subsurface Contaminant Focus Area. However, it is not clear who has the responsibility for "customer service," that is, *assisting the customer with implementation of NABIR products.* While "customer service" is not an explicit goal of the BT and BG program elements, the issue of deliverables is discussed in the Strategic Plan, and it is important for the overall success of the NABIR program that there be a smooth transfer from NABIR to EM and field implementation. With these issues in mind, the Committee recommends that NABIR managers begin to address the 10-year targets that are more closely linked to the needs of the primary customer.

The administrative integration of EMSP and NABIR under a new Division may offer opportunities to address this issue as well as strengthen NABIR science. For example, interdisciplinary collaborations could be stimulated between EMSP researchers involved in modeling and remedial application design with NABIR PIs. This linkage may also promote more interactions with EM, potentially facilitating the adaptation, future field testing, and eventual acceptance of developing technologies among parties responsible for site cleanup. Care, however, must be taken to ensure that the unique focus and long-term research perspective of NABIR research elements be maintained.

2. Are there any areas of research relevant to the BT and BG elements that are not being adequately addressed if at all, and if so, what are they?

While laboratory research on immobilization of metals and radionuclides is underway, studies on the long-term maintenance of the contaminants in immobilized form should be initiated so that tested approaches are in place within the current 10-year NABIR plan. This work should include a measure of the time frame of immobilization reactions *in situ.* Controlled immobilization coupled to removal of the contaminants should be investigated as an alternative to a long-term stewardship, where feasible.

The opportunity should be created for testing immobilization processes under conditions more representative of the real-world environment than a test-tube environment, the artificial condition under which many studies currently are conducted. For example, the effects of bioproducts on the mobility of metals and radionuclides in the subsurface environment need to be explored. Likewise, the effect of extreme conditions that are found in some subsurface environments at DOE sites, e.g., high salinity or extreme pH, may be critical to the implementation of bioremedial strategies developed by the program.

Bioaugmentation is becoming a more feasible remediation approach where indigenous microbes are lacking properties necessary for successful bioremediation. The BG and BT elements should consider this approach. In addition, studies should be conducted on bioaugmented organisms' functioning and survival *in situ*, for microbes whose properties appear to be advantageous.

Most current projects funded under the BG and BT elements do not include a transport element. Instead, research has focused on microscale observations and batch measurements. There is a clear need for studies emphasizing the transport of chemical constituents, microorganisms, and contaminants. Most particularly, the modeling of hydrologic and geologic processes should be incorporated into the biological component to facilitate (a) a more realistic representation of phenomena and (b) the design and evaluation of intermediate scale experiments. Incorporating a transport component will also encourage projects designed to examine the potential influence of subsurface physical, microbial, and geological heterogeneity on the process under study.

Engineering aspects of the implementation of processes developed by BG and BT should be considered to test the feasibility of some biogeochemical approaches developed to immobilize inorganic contaminants.

3. Given that there is some overlap between these two program elements, should they continue to be managed as two discrete, but related elements?

The Committee reviewed the relationship and foci of these two elements as stated in the Strategic Plan and as realized in research activities. The biotransformation element has an appropriate emphasis on the physiology and biochemistry of microorganisms that transform metals and radionuclides, while the biogeochemistry element properly focuses on the characteristics of the biogeochemical reactions. We concluded that both Program elements were thoughtfully and carefully directed and managed, and that significant progress toward Program goals was being attained.

We recommend that the two separate elements be maintained to ensure diversity, creativity, and breadth of scope in the program. The two elements draw different and important expertise to the program, which is important for the basic science objective.

4. How better integrate BT and BG elements with other NABIR elements?

BT and BG projects currently represent excellent basic science but could be strengthened by more of an environmental focus. This strengthening could be achieved by a closer collaboration with community dynamics element investigators. Those investigators need a better understanding of the actual microbial community they hope to emulate in their experiments, including its nutritional status. For example, biomarker analysis can provide information about viable biomass, community composition, and nutritional status. While isolates can be used to define phenomena, a freshly recovered community should be used when possible, e.g. exopolymers formed particularly by Bacilli do not even closely resemble the expolymers encountered in the field. In short, be cautious of inferences about application made from longtime laboratory strains.

Genomics has been a particularly important enhancement to the BT and BG program elements. Detecting motility in Geobacter from flagellar genes in the genome provided new insight on the situ physiology of this organism. Genomics should now provide important insight into gene regulation as it pertains to metal immobilization.

BT and BG raise several potentially contentious issues that should be explored jointly with the BASIC element, including the regulatory and social acceptability of immobilization, confidence that remobilization will not occur, technical and institutional requirements for sustaining immobilization, and confidence in the methods for monitoring the effectiveness of immobilization (detecting or predicting re-mobilization before damage is done).

To facilitate better integration, future calls for BT and BG elements could emphasize integration with other elements, especially community diversity and BASIC, as well as transition to the field and modeling (as discussed under question 6). The Subcommittee noted that the previous integration element, while premature at the beginning of NABIR, may be timely in the future.

5. How can BT and BG researchers take greater advantage of the NABIR Field Research Center (FRC) to understand how to stimulate biotransformations of specific metals and radionuclides in situ at the field scale?

The NABIR FRC offers a number of opportunities for BT and BG researchers, who should be encouraged to take advantage of those opportunities. An obvious advantage of the FRC is its usefulness in representing a set of real-world conditions and contaminants at DOE sites at which bioremediation eventually may be used. In addition, the FRC provides an excellent vehicle for integrating research across program elements—BG, BT, community dynamics, and BASIC. Concentrating multiple research efforts on a single field site can yield a stronger, comparable, and more meaningful set of research results than would come from a disparate set of unrelated field sites. The Subcommittee is pleased that DOE has appointed a Science Coordinator for the FRC, and with the choice of Phil Jardine for that position.

With an eye towards future application, the FRC can be used to test: (a) whether immobilization can be achieved in a heterogeneous subsurface environment; (b) methods for sustaining immobilization over long periods of time (ideally in a way that will prove to be technically feasible and cost-effective); (c) whether methods for sustaining immobilization will have to change over time, as the subsurface environment changes in response to bioremediation activities; (d) conditions that trigger unwanted contaminant mobilization and how to predict and prevent that mobilization in real-world conditions; (e) methods for monitoring the effectiveness of immobilization; and (f) effective nutrient delivery systems.

Nevertheless, alone, the FRC in Oak Ridge is insufficient to meet the needs of the BT and BG program elements. First, not all contaminants of NABIR interest are in the FRC environment. Second, the FRC environment does not represent conditions found at many other DOE sites, especially in the West. Third, and particularly important given the current state of BG and BT knowledge, the FRC is far more complex than the systems under investigation in many BT and BG laboratory experiments. Simpler field environments may provide a more logical “next step” when moving from the laboratory

to the field. Therefore, the subcommittee believes that a limited number of non-FRC sites would benefit researchers engaged in BT and BG work. For example, the subcommittee noted the appropriateness and importance of comparisons presenters made between UMTRA, PNNL, and the FRC sites.

6. How can BT and BG researchers better utilize the results of their basic physiological and/or biogeochemical studies to generate and test predictive mathematical models for use in the field?

BT and BG researchers can better utilize the results of their basic studies in predictive models for the field by involving mathematical modelers early in their projects. In future proposal solicitations, efforts should be made to include modeling expertise in both laboratory and field-based studies. Stand-alone modeling projects would likely be of less value than interdisciplinary, integrated efforts. In the early stages of projects, modelers and engineers can provide insights into the current state of the art on the ability to model the process of interest at the field scale. Modelers can provide input on the limitations that likely will be encountered in scaling-up from microscale laboratory experiments to the field scale. Developing models early in the projects may also help in the design of experiments to be performed. Integrating modeling expertise will help researchers plan experiments that will provide input parameters needed for modeling at the field scale. Experiments can also be planned to assist in model verification.

Involving modelers at early stages of field demonstrations is also important for several reasons. Modelers can: (a) provide important input on parameters values that need to be collected for modeling efforts; (b) help design field tests by incorporating existing models that are likely adequate for determining flow and transport; and (c) provide insight into the uncertainty in the output of the field demonstration using their knowledge of the complexity of the field site. In addition, through modeling, various scenarios can be considered that bound the possible range of process outcomes.

Modeling can also provide insight into the potential performance of a process, even when crude estimates of rates are available. This insight can help determine if a process is feasible for further development. For example, many researchers are discussing plans to create bioreactive barriers. Since NABIR seems to be headed towards this type of treatment process, experimental and modeling efforts should be undertaken to evaluate the practicality of this approach. Among other benefits, modeling can help answer if such systems can be operated for hundreds of years without clogging, or exhausting needed minerals and nutrients.

7. Are BT and BG researchers taking advantage of sophisticated, state-of-the-art DOE user facilities such EMSL, synchrotron radiation sources, and high performance computers; and if not, how might they be encouraged to do so?

NABIR investigators appear to be making good use of the EMSL facility, based on material that appeared in several of the presentations and discussions with those making

the presentations. The subcommittee did not conduct a more systematic investigation of this issue (e.g., examination of number of scientists, projects, publications, etc.) .

EMSL's capabilities in the physical-chemical characterization of the solid phase environment, e.g., minerals, their element composition, and special element distribution, could be combined with *in-situ* molecular characterization of microbial cells and their micromolecules (e.g., FISH). Such integration would enhance the microscale characterization of microbe-metal/radionuclide interactions. For example, such studies could determine what microbes carry out specific biomineralization and biosolubilization reactions, and, possibly, what genes are expressed and enzymes are active during these processes *in-situ.* As the subsurface is largely a heterogeneous microbial habitat, such microscale investigations would enhance understanding of the microbial niche in this environment

To date, high-performance computing (HPC) has played a minimal role in research in the NABIR BG and BT program elements. Funded NABIR projects under the BG and BT elements are now leading to the development of a detailed and mechanistic understanding of the complex and coupled phenomena that influence biotransformation and interfacial reactions. As this research progresses and begins to move towards the testing of technologies at the field scale, the detailed biogeochemistry will need to be incorporated into mathematical models of subsurface flow and transport. These models, if comprehensive, would be extremely computationally intensive for large-scale (site-scale) applications. Thus, HPC could be invaluable to this research program, playing a central role in facilitating the application of such models to the FRC or other field sites. Prior to field scale-up, such models could also be used, in conjunction with HPC facilities, to test hypotheses and examine alternative implementation designs.

NABIR managers should also be proactive in expanding mentoring and collaborative opportunities with DOE lab personnel on the use of toxic and radionuclides, eg. Pu and Am, for immobilization or remobilization experiments that cannot be done at universities. Our concern is that, because of safety and regulatory requirements and the need for specialized facilities and monitoring, a broader group of scientists and perhaps more novel ideas might otherwise be excluded from development.

Another large-scale DOE facility under consideration is the Subsurface Science Initiative at The Idaho National Engineering and Environmental Laboratory (INEEL). This initiative is envisaged to include a large, multi-user intermediate scale facility (http://subsurface.inel.gov/Information/Program/default.asp). This facility might provide conditions missing at the FRC. Our understanding is that this facility is to be operated under the auspices of EM. If this facility is ultimately approved, planned, and constructed, NABIR investigators clearly should be involved, preferably at the planning stage. Notably, there was no mention of this facility during the presentations.

Guidance to NABIR scientists making presentations to the NABIR Subcommittee:

As a general comment, the subcommittee requests that presenters introduce their work by placing it in the context of the NABIR program, not simply the specific program element.

Presenters should indicate why their research is important—how it ultimately can be useful to practical bioremediation application. Because NABIR is a fundamental research program, projects need not have near-term applicability. However, because NABIR also is directed towards actually immobilizing subsurface contaminants, it is incumbent upon researchers to articulate how their work promotes that objective.

Signed for the Committee

James M. Tiedje, Chair, July 10, 2002

Members and guest members present:

Linda Abriola
Tamar Barkay
Enriqueta Barrera
Ed Leadbetter
Lew Semprini
John Westall
David White
Amy Wolfe