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

Community Dynamics and Microbial Ecology Element

Introduction: The NABIR Subcommitee addressed the five questions below regarding program planning for the Community Dynamics and Microbial Ecology (CDME) Element. This element is moderate in size compared to others in the program, and currently contains projects of two primary types: ones developing new molecular methods for community characterization and others focused on understanding the dynamics of microbial communities in contaminated and nutrient-augmented subsurface environments. The Subcommittee understood and supported the need for research in these two subject areas that fall well within NABIR scope as defined in the strategic plan. It was clear that the methods under development could significantly improve the ability to characterize subsurface microbiologic communities in the near future, and the Subcommittee supported the funding of this activity in the early phase of NABIR research. The Subcommittee was impressed by the quality of the science performed and the productivity of the investigators, but it was difficult to recognize the specific component of the research that was NABIR or CDME funded in some of the presentations. Most felt that research in the CDME element could have especially strong contribution to the difficult and unresolved issue of the long-term stability of reduced metals and radionuclides if well conceptualized and conceived. We suggest that consideration be given to additional research on this specific topic and others identified below.

Question 1: Do funded projects support the goals articulated in the **NABIR Strategic Plan** for CDME?

Research in the Community Dynamics/Microbial Ecology (CDME) Element is currently focused on two primary activities that define the first phase of research in this element: 1.) the development of molecular and biochemical methods to characterize microbial communities, and 2.) the evaluation of microbial communities in relevant contaminated environments and those involved in bioremediation. The projects described to the committee from the CDME research portfolio fall into one or two of these activity categories. These two activities clearly support the overall goals of the CDME Element articulated in the strategic plan which are to understand the structure and function of subsurface microbial communities at DOE sites and identify ways to optimize the in-situ growth of microorganisms that transform metals and radionuclides.

It was clear to the committee that significant emphasis had been placed early in the program on the development of new molecular and analytical tools to characterize subsurface microbiological communities. Good choices were made in project selection and impressive, exciting, state-of-the-art capabilities are emerging with clear usefulness to the NABIR program and the scientific community generally. The project mix appears excellent given the funding availability, and the characterization tools that will result are diverse in the phenomena that they target and information that they will provide. This

diversity well serves the needs of the broad-based NABIR microbiological research community. The rational that these tools were needed before significant progress could be made in characterizing subsurface microbiologic communities before and after biostimulation was clear to the committee.

The existing community dynamics research portfolio, in contrast to the methods development activities, was viewed to support the strategic goals only in part. The CDME Element currently contains one or more projects that predate the NABIR Strategic Plan, and that are not well aligned with it. For example, the contaminants and sites being studied in at least one of these projects has questionable merit in terms of its relevance and scientific applicability to the DOE lands that are the program focus. DOE sites facing remediation may exhibit unique or specialized microbiologic and biogeochemical characteristics because of their geographic location, hydrologic/geohydrologic regime, operational history, and chemical nature of the waste stream (sometimes extreme) in terms of contaminants, radioactivity, co-contaminants, salts, carbon compounds, potential nutrients, and oxidizable/reducible substrates. Accordingly, the panel felt that the study of microbiologic community structure, dynamics, and biogeochemical function on DOE lands was lagging. CDME could better support the strategic plan by placing more emphasis on the study of representative contaminated sites on DOE lands to the extent that site access and environmental health issues allow.

Question 2: Are **relevant areas** to being adequately addressed?

For the most part, the panel felt that the important research questions in this overall scientific area were or will be adequately addressed in the future if the strategic plan is followed and funded to requested levels. The panel did, however, identify a number of research areas both germane, and arguably critical to NABIR research objectives that might be considered by NABIR Management.

1. The current emphasis in other NABIR research elements on SRB and DIRB (including pure culture work employing *Shewanella spp, Geobacter spp, Desulfovibrio spp*) systems is well justified because these organisms mediate the reduction of soluble, polyvalent metals and radionuclides to insoluble oxide forms. However, the NABIR research objective for long-term, in-ground immobilization requires that other types of microorganisms and communities with different function be studied and manipulated for their ability to influence and/or control the long term stability of oxygen-sensitive reduced contaminants (e.g., U(IV), Tc(IV)) Yet, CD/ME studies with model systems with different dominant electron acceptors (e.g. O2) may need to be considered if, e.g. long term fate of elements in the vadose zone is under study.

2. The panel felt the CDME research focused on community dynamics could benefit through collaboration with investigators of geochemical and hydrologic expertise. Obviously the dynamics of subsurface microbial communities are influenced by many complex factors. Nutrient and contaminant flux, geochemical factors, and

microphyscial aspects of the porous/fractured media, however, are widely recognized to be important but seem to draw little NABIR research attention. Along these same lines, it was felt that studies on the impact of contaminant chemical speciation on bioavailablity and community dynamics would be fruitful. Chemical speciation and its effects on contaminant and mineral transformation is being studied in both the Biogeochemistry and Biotransformation Elements, and extending that concept to CDME could serve to integrate research between these elements. It was also recommended that experimental studies on the effects of key physical, chemical, and hydrologic factors be studied on the community dynamics of subsurface materials from relevant sites in controlled laboratory settings.

3. All agreed that the program could benefit from additional research on the details of microbiological community dynamics, especially during and after biostimulation. While it was noted that such research was planned in future years of the NABIR program, detailed studies of this nature provide critical information to the viability of the remedial concept being developed by the program. For example, the acetate injection experiment that was described at the Old Rifle UMTRA site presented an interesting case of community evolution from Fe(III)-reduction to sulfate reduction as the electron acceptor (Fe(III)) oxide became limiting (apparently). This rapid shift in community function lead to decrease in U(VI) reduction and immobilization that compromised the remedial function of biostimulation. This interesting case study provoked unresolved discussion on the best ways to control the community function (in this case Fe(III) reduction) along desired pathways for remedial goal (e.g., continued enzymatic reduction of U(VI)). Overall it was felt that the program could benefit, now, from accelerated research on subsurface processes controlling community dynamics and well conceived laboratory and field studies of species competition before, during, and after biostimulation.

4. This final suggestion is repeated in responses to other questions. The NABIR program could benefit through the characterization of microbiologic communities in representative DOE Sites containing, for example: complexed radionuclides, mixed contaminants including actinides and transuranic, co-contaminant metals and organic compounds, extremes in pH, and elevated thermal regimes. The panel did not necessarily recommend that new projects be specifically initiated to accomplish this task. It was noted that ongoing contaminant and hydrogeologic characterization activities at a site like Hanford often provide as yet unutilized opportunities to investigate the microbiology of some truly unique, high visibility, contaminated sites. One such opportunity will occur in early spring as Hanford cores beneath leaked high-level waste tank T-106 where a massive vadose zone plume exists with an unusual chromatographic pattern of actinides and other fission products. Perhaps the program should consider some type of response team with individuals from several projects that have interest in the characterization of these types of contaminated lands. While these activities may fall outside of the current NABIR scope, information might accrue that could lead to exciting new directions for NABIR research.

Question 3: How can CDME to **better integrated** with other elements?

The ultimate goal of the NABIR program is to achieve the immobilization & long term stability of key contaminant metals and radionuclides on DOE lands through stimulation of subsurface microbiologic communities. DOE lands exhibit specific climatic, geologic, geochemical, hydrologic, and waste characteristics that may exert strong influence on the nature and composition of subsurface microbiologic communities present at these sites. Accordingly, it is important that CDME projects (some but not necessarily all) focus on microbial communities, contaminant suites, subsurface conditions, and scientific issues that are truly relevant to the problems that the program is seeking to resolve. In order to achieve such relevance both the investigators and NABIR program management must understand, to some degree, the geohydrochemical characteristics of the big DOE sites and their typical patterns of contamination. The panel did debate the question as to how much DOE site alignment was necessary in a given project to develop the desired scientific understanding of subsurface microbiologic processes. While not entirely resolved, the panel felt that more, as opposed to less, DOE site alignment would facilitate the attainment of the articulated program goals. Achieving this focus will require some project realignment, but the resulting portfolio will be complementary with other NABIR projects and research elements through common emphasis of a single suite of DOE contaminants.

Increased integration of CDME research with other elements could be achieved by specific articulation of this intent in future NABIR calls, or by having element-specific breakout sessions at the annual meeting that both encourage and direct collaborations with researchers from other elements. The objective would be to have CDME investigators seek out investigators from other elements, and the reverse as well. A new NABIR call, for example, could emphasize that i.) CDME research be performed at the FRC, or other specific DOE sites identified by the program, or ii.) that biotransformation or biogeochemical research utilize communities or metabolically specific enrichment cultures characterized or isolated by CDME , or iii.) that specific new community characterization tools developed by CDME be applied in FRC field experiments or in biogeochemistry/biotransformation studies with DOE sediments. Another thought might be to encourage, in future NABIR proposal calls, a limited number of larger, high impact, multi-institutional proposals that span, link and integrate research through several NABIR elements.

Question 4: How can CDME researchers take better advantage of **field sites**?

The NABIR Field Research Center (FRC), UMTRA sites, and other sites provide unique opportunities for researchers to apply assessment methods being developed in this element. Many of the methods that are being developed can be readily incorporated into experimental plans for field research. Researchers did indicate that these collaborations were occurring, however their extent was difficult to assess. The NABIR program has facilitated these collaborations through planning workshops. Development of a long-term plan for the use of methods that have been developed would facilitate research

involvement at the FRC and UMTRA sites. Researchers should be encouraged to integrate their assessment tools into projects where relevant processes are being investigated and where an understanding of community dynamics is important. For example, attempts to immobilize uranium by stimulating iron reducing bacteria, which are affected by processes utilizing other terminal acceptors, could be coupled to community analysis in the remediated site. By working at the site a collaboration of geologists, hydrologists, microbiologists, and geochemists will be enhanced and in turn will enhance achieving the program's goals. Employing established methods of assessment for microbial community analysis for these sites of interest, along with innovative methods, such as future community genome arrays and functional gene arrays, should be encouraged. Future request for proposals should stress the advantage of using methods that have been developed within this program element as well as elsewhere for application at the FRC and UMTRA sites.

Question 5: Is the investment in **new methods development** appropriate? Should **other innovative methods** be explored?

The investment in 'new methods development'' (or 'Assessment') includes projects focused on development and use of DNA micro array technology, lipid biomarker analysis, genome sequencing and gene expression analysis. The investment and focus of projects in this sub-element was appropriate and very successful at meeting the goals of the initial Phase of the CDME element. While fundamental work in tool development is still needed, future focus should emphasize application of the methods to support other NABIR efforts that are underway or planned at DOE sites.

Certain of the methods currently supported by NABIR are of great interest to other parts of DOE and to other agencies (within a context broader than environmental soil and groundwater sampling, e.g. micro arrays for biomedicine, seawater sampling, wastewater sampling). It is expected that these entities will continue to fund the fundamental aspects of method development (e.g., DNA attachment chemistry for micro arrays, phylogenetics, algorithms for designing primers/probes). Some of this research and development is quite costly. Therefore, NABIR should consider focusing some if its limited resources on projects that are seeking to apply current and evolving methods to DOE-site-oriented projects (FRC, UMTRA) in support of NABIR science goals. It might also benefit the investigators who are developing and optimizing new methods to partner with projects that are more hypothesis-driven. The use of the lipid biomarker analysis method to support microbial ecology projects at the UMTRA sites as well as push-pull studies at the FRC is an excellent example of how these types of efforts can mesh extremely well to support and advance our knowledge of "the potential for natural microbial communities to immobilize metals and radionuclides" (NABIR Strategic Plan).

It was also a conclusion of the panel that one of the CDME 3-year targets, "To determine the importance of gene transfer within microbial communities involved in bioremediation" (NABIR Strategic Plan), is perhaps not being adequately addressed, and that more significant investment in this area is warranted. Little is known about the

spatial and temporal aspects of this phenomenon under environmental conditions, yet understanding factors that control and influence gene transfer may yield exciting and useful information for NABIR and DOE.

Signed for the committee:

John M. Zachara, Chair, November 26, 2002

Members and guest members present:

Tamar Barkay
Margaret Cavanaugh
Linda Chrisey
Lew Semprini
Barth Smets
Mary Voytek