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ENVIRONMENTAL BIOTECHNOLOGY OF HAZARDOUS WASTES

Research Planning Workshop
The National Science Foundation

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FOR THE UNITED STATES
DEPARTMENT OF ENERGY

Printed in the United States of America. Available from
National Technical Information Service
U.S. Department of Commerce
5285 Port Royal Road, Springfield, Virginia 22161
NTIS price codes—Printed Copy: A03; Microfiche A01

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Date Published - August 1988

NSF Project #CES-8714691

Prepared by the
OAK RIDGE NATIONAL LABORATORY
Oak Ridge, TN 37831
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MARTIN MARIETTA ENERGY SYSTEMS, INC.
for the
U.S. DEPARTMENT OF ENERGY
under contract DE-AC05-84OR21400

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PREFACE

The potential exists to use native or genetically engineered microorganisms for the control of hazardous wastes and environmental contaminants. However, the development and application of engineered organisms and their native wild-type counterparts for predictable and effective control of hazardous wastes has only been achieved in a rudimentary sense. To date, the root causes for this failure are multifaceted and related to the adequacy of the technology knowledge base, integration of responsible science and engineering disciplines, and existing technology policy.

In response to these problems, the National Science Foundation Engineering Directorate sponsored a research planning workshop to define current limitations in the development of environmental biotechnology for hazardous wastes and to develop a consensus basic research agenda and plan. An expert working panel, representing the broad science and engineering disciplines contributing to the development of environmental biotechnology, met October 30 through November 1, 1987, in Gatlinburg, Tennessee, to develop this agenda and research planning strategy.

ABSTRACT

Environmental biotechnology is "the direct use of microorganisms and their capabilities to solve environmental problems and for in-situ agricultural applications and industrial waste treatment." Environmental biotechnology is at the interface between responsible disciplines in engineering, molecular biology, and ecological sciences.

The purpose of this research planning effort was to define limitations in the current knowledge base and to develop a research agenda and strategy that will lead to the successful practice of environmental biotechnology. The focus was specifically directed at environmental protection. However, it was recognized that the research plan typifies a central research model for the future development of effective and ecologically sound biotechnology for other environmental applications.

The panel members (Appendix A) agreed that the present limitations in the development and implementation of environmental biotechnology are attributable to a number of factors:

1. The present knowledge of the biochemistry, genetics, and ecology of biodegradative microorganisms and genes is limited to a few well-characterized model systems of unknown relevancy to applications in open environments and engineered systems.

2. Attempts at strain improvement by conventional selection and genetic engineering techniques are largely limited in their scope, relative to the needs for application to real problems.

3. Engineering applications for biodegradative microbial populations are generally limited to relatively easy-to-degrade chemicals under relatively ideal conditions.

4. Specific and quantitative analytical and molecular tools are needed to monitor and control the population dynamics and activity of biodegradative organisms and genes.

5. Integration of the required disciplines in problem-identification and problem-solving modes has not been achieved.

6. Available resources to support the needed fundamental research contributing to environmental biotechnology are insufficient and scattered among diverse sources and are not targeted to a strategy that will result

in the timely development and implementation of the technology for the control of hazardous chemicals.

A strong consensus strategy for development of environmental biotechnology was reached during the workshop. This strategy can be implemented through:

1. An interdisciplinary research agenda, which should include four major program elements:
   - agent and strain development and improvement,
   - development and improvement of analytical and molecular monitoring methods,
   - environmental and ecological systems analysis, and
   - engineered systems analysis.
2. Single-investigator and multi-group research efforts. These efforts should be targeted to the long-range research needs of environmental biotechnology.
3. An effective interdisciplinary peer review system, which must accommodate the unique aspects of interdisciplinary research and be committed to the research agenda in a manner that will be competitive with traditional research programs.

1. INTRODUCTION

Environmental contamination is a problem of global concern. Since 1950, possibly six billion tons of hazardous wastes have been deposited in or on the land.[1] A report by the Congressional Office of Technology Assessment (OTA) estimated that from 255 to 275 million tons per year of hazardous wastes under federal and state regulations are being disposed on land.[2] In recent times, and with increasing frequency, hazardous waste sites have achieved national notoriety (Love Canal in New York, Times Beach in Missouri, Stringfellow Acid Pits in California, Rocky Mountain Arsenal in Colorado, etc.). Reports of groundwater contamination have occurred in all 50 states, and several thousand public and private wells have been closed. Organic chemicals are the major problem; for example, trichloroethylene has been found in one-third of the cases of contaminated groundwater associated with hazardous waste sites.[3]

Major laws affecting the management of wastes have been enacted. The most notable are the Resource Conservation and Recovery Act of 1976 (RCRA); the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA, popularly known as Superfund); and the Hazardous Solid Waste Act of 1984. These acts have been amended and reauthorized, generally with increased requirements and appropriations as the increased extent of the problem is recognized. Under the Superfund legislation, the U.S. Environmental Protection Agency (EPA) developed the National Priorities List (NPL), which identifies the nation's most hazardous sites requiring cleanup action. EPA estimates that the NPL will grow to 2,000 sites, while the OTA estimates that the number of sites requiring Superfund cleanup could be 10,000 or more.

The cost of cleanup will be an enormous drain on the nation's economy and will directly impact the international competitive position of most of our country's industries, including agriculture, electronics, chemicals, and pharmaceuticals. It is projected that by 1990, implementation of Superfund and other waste-related regulations will cost U.S. industry $20 billion per year.[4] Cleanup of the NPL sites, alone, may require several hundred billion dollars and 50 years.[5]

Cleanup avoidance and relaxation of waste management regulations will not be socially acceptable options. Considering the high cost of cleanup and the potential threats to human health and the environment, the proper task of the technical community is to develop acceptable and effective technologies to accomplish socially-mandated cleanup and restoration of the environment. Actual destruction of chemical wastes is conceptually preferable to physical isolation by any technology. In some cases, incineration is a potential hazardous waste management technique; however, it is expensive.[5]

Biodegradation offers a very attractive option for destruction of many hazardous wastes. Genetically engineered microorganisms and indigenous organisms isolated from natural sites may offer great specificity and efficiency for mineralization of organic compounds.[6–8] In-situ treatment is often a realistic possibility. These advantages are widely perceived to be relatively inexpensive.[5] Costs are sometimes estimated by private sector contractors to be only 10 to 50% of costs for alternatives such as incineration or dig-and-haul. However, these estimates are derived from the current inefficient technology and may be further reduced by additional research.

Also widely recognized in the waste management community is the fact that reliable, practical technologies for biodegradation do not exist. Occasional success stories are reported and interest remains high.[9] Recent symposia on waste treatment have frequently included sessions on biodegradation of hazardous wastes, especially in-situ technologies. Substantial technical progress in both in-situ technologies and in bioreactor systems is hampered by the relative lack (to date) of microorganisms for specific applications; the absence of a reliable protocol for process design, scaleup, and optimization; and concerns over the consequences of release of genetically engineered microorganisms to the environment. The potential capacity of microorganisms to degrade a variety of xenobiotic and recalcitrant wastes has been described in numerous reviews,[10–13] but exploitation of this vast degradation potential has yet to be realized.

2. ENVIRONMENTAL BIOTECHNOLOGY

Over the past two decades, developments in recombinant DNA technology have promoted a virtual explosion of research and new knowledge in modern molecular biology. The rapid development of this field is the result of scientific breakthroughs allowing the controlled modification, introduction, and expression of foreign or native genes in a host organism. The recombinant DNA technology used to reach these achievements was the subject of serious scientific and public concern over the risks and ethics of such "genetic engineering." These concerns and their possible impediments to future research were expressed at numerous scientific forums, perhaps best exemplified by the Asilomar Conference,† and led to the eventual establishment of the Recombinant DNA Advisory Committee (RAC) and the National Institutes of Health (NIH) guidelines for recombinant DNA research. The flexibility and evolution of the NIH guidelines, in response to an expanding knowledge base of the limitations and safety of recombinant DNA technology, and have been largely responsible for the structured growth and transition from basic and applied research to the development and use of living organisms and their parts and processes to benefit mankind. Opportunities abound for use of "tailor-made" microorganisms and their protein products in product-oriented areas such as health care, agriculture, commodity and specialty chemicals, and environmental protection.[14]

The direct use of microorganisms and their capabilities to solve environmental problems and for in-situ agricultural applications and industrial waste treatments can be defined operationally as environmental biotechnology. Applications include detoxification and/or destruction of pollutants and hazardous wastes, improvements in soil fertility and crop productivity, biological pest management, and restoration and renovation of perturbed ecosystems. Environmental biotechnology is differentiated from other areas of biotechnology in that successful process development must contend with the complexity of mixed (heterogeneous) populations and

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†International Conference on Recombinant DNA Molecules, Asilomar Conference Center, Pacific Grove, California, February 1975.

interactions occurring in ecosystems and by the fact that the technology, itself, may be the subject of concern over environmental hazards. Consequently, there are major needs for both ecological and engineering research to enable utilization of modern applied molecular biology for successful process development and overall risk assessment.

In spite of the tremendous potential for use of microorganisms in the environment to accomplish desirable objectives, current practice of environmental biotechnology is relatively primitive:

1. Applications are limited to relatively easy-to-degrade compounds, such as hydrocarbons, under relatively ideal conditions.
2. Availability of microbial cultures for degradation of xenobiotic (man-made) compounds is low.
3. Ultimate fates of contaminants frequently cannot be assessed.
4. Laboratory studies cannot be reliably scaled up to field applications.
5. Field-scale biotreatment processes are poorly characterized and poorly understood.
6. Predictability of process performance is low.
7. Process performance is frequently poor and disappointing.

These limitations have been discussed in recent reviews of biodegradation in groundwater and in activated sludge systems.[15-18] The source of these limitations is not just inadequate understanding of biodegradation; often, the sites are extremely complex chemically, physically, and biologically. These realities will also need to be addressed through appropriate research in order to improve the practical utility of biodegradation technologies.

There is little doubt that recent developments in molecular biology and recombinant DNA technology have stimulated new interest in biodegradation. It is now possible to plan research to genetically modify microorganisms to expand the range of substrates degraded and to increase the rates at which degradation occurs. Several recent workshops and symposia have been devoted to this opportunity.[19-23] These forums have highlighted two principal needs:

1. The need for more fundamental information on the occurrence, biochemistry, genetics, and ecology of biodegradative

microorganisms and genes for specific environmental contaminants; and

2. The need for an interdisciplinary research infrastructure that integrates the molecular biology, biochemistry, microbial ecology, and engineering sciences that are required for developing practical biodegradation technologies.

Environmental biotechnology, as defined in this report, provides the framework for such an infrastructure at the interfaces between the traditional science and engineering disciplines.

Although interdisciplinary collaborative research is frequently stimulating and rewarding to the investigators, institutional behavior and barriers are often a factor, as pointed out in a recent OTA report[24] and also by the National Research Council, which said:[25]

> **"Interdisciplinary research between physical-engineering sciences and biological-clinical sciences has produced important new knowledge and medical advances, including research and diagnostic instruments such as CAT scans and permanent implant devices such as cardiac pacemakers and joint replacements. Applying the knowledge of how biological systems are designed promises to result in more refined and powerful techniques to further basic understanding and to treat living organisms.**
>
> **But there are some major impediments to interdisciplinary collaborations. Differences in conceptual approach may create language and communications barriers. Lack of career incentives and rewards due to institutional and organizational constraints also may interfere. And formal training and orientation are not the same among practitioners of different disciplines. Because of these differences, scientists may be unaware of how and when to capitalize on the benefits to be gained by collaborative research."**

3. RESEARCH AGENDA

Interdisciplinary research, as described in Figure 1, representing microbial biochemistry, genetics, ecology, environmental microbiology, and chemical and environmental bioprocess engineering is needed to develop practical environmental biotechnology. It is also important to ensure that this option is successfully integrated in the overall strategy of process development for management and control of hazardous wastes and environmental contaminants. The goal of environmental biotechnology research is to provide current and future direction for integrating modern biotechnology, environmental science, and engineering research for the control of hazardous wastes and environmental contamination.

ORNL DWG 88-1024

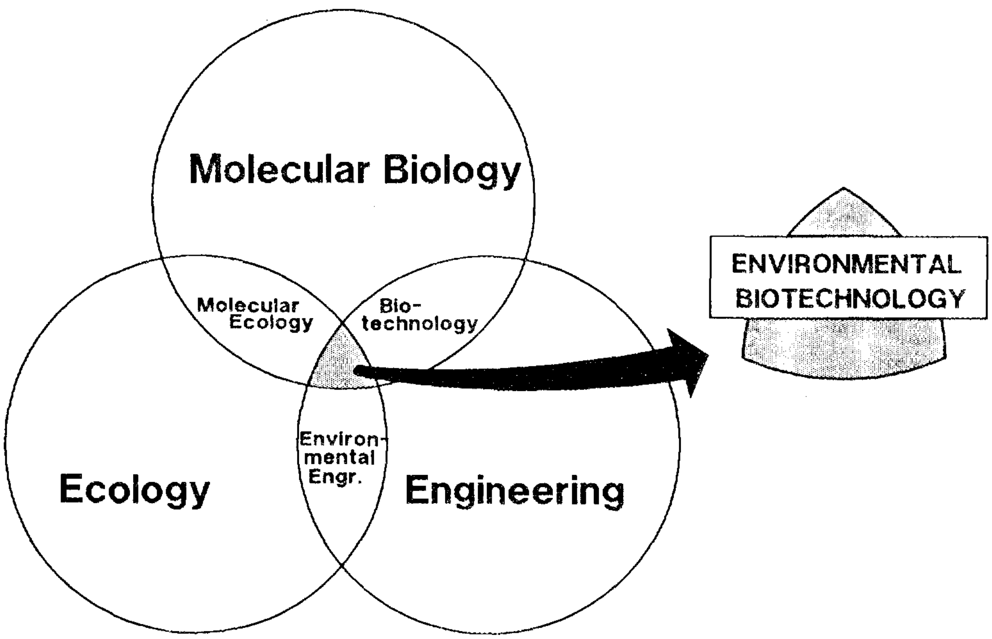

Figure 1. Integration of science and engineering disciplines to create environmental biotechnology.

The workshop participants agreed on an agenda for fundamental research in environmental biotechnology. The four major program elements are outlined as follows:

Agent (Strain) Development

- Source and Selection
- Characterization
- Modification and Improvement
- Model Systems (mixed and pure)
- Evolutionary Relationships/Diversity
- Stress Response
- Collections and Libraries

Process and System Analytical Tools

- Quantitative Analytical Techniques (Chemical/Physical Measurements)
- Bioanalytical Methods
- Molecular Analysis Methods
- Remote Sensing
- Biomonitors
- Reporter—Signal Analysis/Structure and Function

Environmental System Analysis

- Ecological Interactions
- Environmental Fate and Abiotic Processes
- Population Dynamics (organisms and genes)
- Environmental Stability
- Determination of Kinetic Parameters
- Microhabitats - Niche Invasions
- Organismal or Genetic Mobility
- Controllability and Environmental Modification
- Stress-Induced Effects

Reactor System Analysis

- Reactor Design
- Transient Outcomes and Perturbations
- Dynamic Analysis
- Ecological Interactions
- System Stability and Component Stability
- On-line Analysis and Control
- Kinetic Parameters Analysis

Microbial strain development and improvement includes the isolation and characterization of new and existing biodegradative strains, as well as the molecular and biochemical characterization, modification, and development of strains with ancillary properties that are useful for control and optimization in potential field applications. Research in this area needs to exploit the genetic and biochemical versatility that microorganisms have evolved in natural, stressed, and exotic environments. Major opportunities exist to develop new information on novel or alternative molecular and biochemical systems important in biodegradation and in organismal and gene maintenance in the environment. The resulting information base and the organisms obtained with this research should be broadly available to the science and engineering community in the form of specific data bases and a library collection of available strains.

Advanced analytical approaches are required for facilitating the detection, quantitation, isolation, and monitoring of microbial agents for control of hazardous wastes and environmental contaminants. The long-term success or failure of applications of environmental biotechnology is dependent on the ability to monitor and control the process to demonstrate efficacy as well as safety. State-of-the-art instrumental and molecular methods for specific and sensitive quantitation of individual organisms and genes need to be further refined and automated to permit rapid analysis of abundance and activity in complex microbial populations.

Environmental and engineered or reactor systems research focuses on the complexity of mixed biological communities and the often heterogenous and variable nature of the physical and chemical parameters describing the system. These research elements ultimately describe the limits, or regimes, to which the biotechnological product or process is expected to function. Furthermore, laboratory findings on the stability, evolution, and transfer of recombinant genes can only be made meaningful on a quantitative basis through adequate environmental simulations.

Risk assessment for environmental biotechnology was not considered at this workshop. Increased technical understanding gained through research discussed previously will contribute to the data base needed for the assessment of specific uses of environmental biotechnology.

4. STRATEGIC ISSUES

1. It is most likely that the major advances in field applications of the technology will be the result of interdisciplinary research that can be scaled from the lab environment to complex engineered systems and the open environment.

2. Targeted individual investigator research is desirable in all areas of the research agenda. The direction of this basic research should be toward expansion of the knowledge base and biological resources that will contribute to application of the technology.

3. Targeted research is needed to exploit specific applications of the technology. Research to expand the range of degradative organisms available, the development of model systems and analytic tools, and reactor and environmental systems design should be identified jointly by the responsible disciplines.

4. Training of technologists and policy makers will encourage a more rapid pace at which environmental biotechnology will be mainstreamed into the national science and technology agenda and transferred to the marketplace.

5. A peer-review and project-selection process is needed that will accommodate the unique characteristics of interdisciplinary research. An aggressive, positive system is needed that will recognize the innovative aspects of research at disciplinary boundaries and will compensate for the parochial tendencies of reviewers to downrate proposals that include research not in the mainstream of the reviewers' interests and expertise. This system must be competitive with traditional existing peer review and project selection in order to obtain a fair share of limited resources.

6. Funding levels must be adequate to support multiple investigators, when necessary.

5. RECOMMENDATIONS

The research needs and strategic issues can be addressed through various mechanisms. Following is a summary of the particular approaches favored by the workshop participants. All of these approaches are potentially viable and are consistent with present activities in the NSF:

1. individual projects funded through existing program areas;
2. individual projects co-funded through several existing program areas;
3. centers of various sorts, large and small; and
4. new program area, either within an existing directorate or perhaps an interdirectorate program administered by Engineering and Biology.

Funding considerations may favor organizations that obtain funding from several existing program areas. In this age of little or no-growth budgets, co-funding may be more practical than carving out a large new budget area. However, because of the significance of the research area, management decisions could be made at the appropriate levels to create a budget for a new program. The workshop participants recommend funding for research in this area at a minimum of $5 million per year.

The consensus was that the particular organizational home within the NSF is not the critical element for a successful interdisciplinary research program in the use of microorganisms for waste treatment and remediation of environmental contamination. Rather, there are three critical features that can and should be embraced by whatever structure is adopted.

1. The program should actively solicit interdisciplinary collaboration. The more apparently diverse the disciplines, the better, as long as there is evidence of bona fide collaboration. Two or more separate but related projects are not sufficient in most cases of individual projects. (Center programs would be exceptions.)

2. The program must have a proposal review and evaluation process that enables interdisciplinary proposals to receive outstanding ratings in order to compete internally for funding. Such a process may need to be different from the traditional NSF review and evaluation process in, perhaps, minor but significant ways.

3. Funding for each project should be adequate to allow the needed inter-investigator collaboration. It is not reasonable to expect first-

class investigators to collaborate free (i.e., when the funding level is adequate to support only one investigator).

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