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Project Summary

Genetic Engineering and the Development of New Pollution Control Technologies

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This report relates genetic engineering to biological waste treatment, so that opportunities for its improvement can be identified and evaluated. It compares the present (mid-1983) state of development of gene manipulation and natural limits to biodegradation.

It identifies a number of research topics that are likely to contribute to new pollution treatment techniques. These topics include the basic mechanisms underlying microbial co-metabolism and oligotrophy; molecular genetics in filamentous fungi, in strict anaerobes and in archaebacteria; directed evolution of enzymes and metabolic pathways; and studies to advance understanding of dehalogenations by microbes.

This Project Summary was developed by EPA's Industrial Environmental Research Laboratory, Cincinnati, OH, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

The objectives of this study were to document the basis for developing new pollution controls using genetic technology, to describe the present state of such development, and to recommend a research policy for such an approach. The study was performed by assembling three review papers dealing with pollution problems, gene manipulations, and natural limits to biodegradation. A panel of experts representing disciplines that might contribute to the development of new treatment technologies assembled in Urbana, Illinois, to discuss these papers. Their recommendations were merged with the information gathered in the background papers to provide a sound basis for recommending policy.

It was generally concluded that the spectacular recent advances in gene manipulation afford a remarkable new opportunity to design and create microorganisms with defined, desirable biodegradative capacities. But it was also concluded that this ability represents only one of the necessary conditions for the creation of new and practical pollution abatement processes. The other necessary condition is to bring a population of microorganisms having a suitable amount of a relevant catabolic potential into contact with a pollutant. This involves either establishing a new population of microorganisms in a polluted environment or distributing the genes for a biodegradative pathway among the members of an existing microflora at a polluted site.

Basic knowledge of these two processes is fragmentary compared to knowledge of gene manipulation technologies. Consequently, the primary recommendation of the study is to support basic research on organism establishment and gene transmission in natural microbial populations. This recommendation states that the establishment of microbes, or their genes, in polluted matrices is as important to practical treatment processes as the creation of microbes with novel biodegradative capacities.

Release to the environment of engineered microorganisms was identified as an issue of concern to the public and to bodies such as the Recombinant DNA Advisory Committee (RAC) that is charged with the oversight of some of the new genetic technologies. To allay fears and to ensure a prudent and responsible development of new biological pollution treatments, it is recommended that experiments involving the release of genetically manipulated microorganisms be reviewed and approved for environmental safety by a scientifically qualified public body. When possible, organisms destined for release should be constructed without the use of *in vitro* recombinant DNA. This should expedite approval of release, since all techniques except the *in vitro* recombinant DNA method are currently viewed as essentially natural and innocuous.

Two conventional treatment problems that might benefit from exploration from a genetic perspective were the removal of ammonia by low-aged wastewater sludges and improvements in the flocculation of these sludges. Unlike research on organism establishment and gene transmission in indigenous populations, no priorities were assigned to these recommendations.

The study recognized that the advancement of waste treatment through genetic manipulations will require the involvement of scientists in a very broad range of disciplines. Even in assembling the background papers for this study, communication among scientists was impeded by differences in disciplinary perspectives, in terminology, and, most importantly, by a poor understanding of the fundamental capabilities and limits of the essential contributing disciplines. A number of measures were recommended to improve communication among the specialists in the contributing sciences. These included support for problem-focused symposia, an occasional newsletter, the coordination of research support among government agencies, and a listing of pollution problems, research needs, and current developments.

Results

There was general agreement among the participants at the Urbana workshop regarding some aspects of the development of new biological pollution control agents. It was generally held that this approach offers a great deal of promise. The following outline of conclusions and recommendations provides a good sketch of such promise and recommended research.

Outline of Conclusions and Recommendations

I. Release of Engineered Organisms for Pollution Control
   - A. Concern about the release of engineered organisms demands the experiments involving their release to the environment be reviewed and sanctioned by a scientifically qualified public body.
   - B. Current regulations governing the release of microbes altered by *in vitro* recombinant DNA methods are not a barrier to the development of such organisms, although release to the environment is regulated.
   - C. Development of new organisms by *in vivo* recombination and other techniques not now regulated should be emphasized.
   - D. The possibility of exceptional examples of potentially hazardous strains arising during the development of pollution control agents by purely unregulated manipulations cannot be ruled out at present.

II. General Conclusions
   - A. Genetic engineering offers promise for new pollution treatments.
   - B. Single strains degrading a categorical collection of pollutants (e.g., PCBs) will not be found.
   - C. Compound-specific and site-specific treatments must be emphasized initially.

III. Recommended Research
   - A. Fields
     - i. Colonization of polluted environments by introduced microbes
     - ii. Gene transmission in the environment
     - iii. Co-metabolism
     - iv. Oligotrophy
   - B. Areas
     - i. The genetics of catabolism by archaebacteria
     - ii. The genetics of catabolism by anaerobes
     - iii. The genetics of catabolism by filamentous fungi
     - iv. The genetics of microbial dehalogenation
     - v. Artificial evolution of enzymes and metabolic pathways
   - C. Projects
     - i. Flocculation in wastewater sludges
     - ii. Ammonia removal by low-age wastewater sludge

IV. Implementation
   - A. Knowledge from relevant fields should be developed in parallel and integrated through a newsletter and problem-focused symposia.
   - B. Research undertaken by the USEPA and other government agencies and organizations should be coordinated.
   - C. A current listing of relevant scientific developments, pollution problems, and research needs should be compiled, distributed, and updated regularly.

Although some aspects of this new pollution technique appear promising, several cautionary notes were sounded. An obvious caution was that pollution problems must be clearly defined in terms of their relevance to genetics and biodegradation before potential solutions can be discussed. Seemingly self-evident, this notion is violated by suggestions such as the creation of "superbugs" to reduce the BOD of an effluent or the volume of sludge from an aeration basin. Genetic engineering most often involves the specific alteration of individual biochemical pathways in particular organisms. Individual strains with specialized pathways can have but little effect on the uncharacterized and presumably heterogeneous organic matter measured by the BOD test or on the organic matter present in wastewater sludge.

Similarly, a caution was expressed about the prospects for developing single organisms able to attack pollutants such as PCBs or toxaphenes that are actually collections of diverse substances. It was pointed out that bacteria frequently attack only specific members of a set of related compounds. Thus, differences as small as a single hydroxyl substitution, for example in benzoic, *p*-hydroxybenzoic and salicylic acids, are discerned by bacteria. Usually such compounds are degraded by separate strains using wholly separate pathways. A single bacterial strain will frequently degrade compounds related as intermediates in a catabolic pathway, for example, naphthalene, salicylate, and catechol, more readily than a set of compounds with close chemical relationships, for instance, the position isomers, phthalic and terephthalic acid, or a set of compounds with varying chemical substitutions at one position, such as chlorobenzene, phenol, aniline, nitrobenzene, and toluene. This apparently stems from the generally high specificity of enzyme-substrate interactions and from the need to foster efficient degradation by integrating the products of catabolism into the central metabolism of an organism.

Apparently exceptions to this specificity do exist. Some oxygenases occurring early in a degradative sequence show a more universal appetite than is usual for typical enzymes of intermediary metabolism, for example, benzoate oxygenases that can accept chlorobenzoates as substrates or naphthalene oxygenases that can utilize methyl naphthalenes. Further, it has been pointed out that many catabolic pathways tend to converge on a few common metabolites, for example, aromatic degradations converging on catechol or gentisate. Nevertheless, these exceptions to absolute specificity are not a sufficient basis for designing an organism to attack so broad a categorical set of compounds as the PCBs; it must be anticipated that a large group of specifically developed bacterial strains, each attacking one or only a few members of the set, will be needed to achieve a comprehensive attack on collections of compounds like these.

Another caution noted was that compounds rarely occur singly in nature. Similarly, pollutants will most commonly be found as components of a mixture of compounds. The other compounds occurring in conjunction with a target pollutant might be either beneficial or detrimental to the organism degrading the pollutant. In addition, the physical conditions present at the polluted site will individually and perhaps synergistically alter the functioning of any given engineered microbe. Thus, a strain used at one site would not necessarily work well at another, even if both sites contain a common target compound. This would be due to the nature of the chemical mixture or the environmental conditions present at each site. Thus, at least initially, each pollution problem should be addressed site-by-site, pollutant-by-pollutant. Ultimately, it may be possible, after achieving improvements in treatments of some specific pollutants, to identify groups of sites with sufficiently similar characteristics that a particular manipulated organism can be used at all similar sites. The initial experimental work, however, should be done with a narrow focus to provide a foundation for what will follow.

Conclusions and Recommendations

Presently, the strengthening of research in a few specific subjects appears to offer the most expeditious means to promote the genetic engineering approach to pollution control. The recommended research outlined above varies in scope from specific projects to broad fields of study.

The establishment of introduced organisms in the environment and the transmission of genetic material among indigenous microflora are the two steps basic to every suggested improvement in pollution control through genetic engineering. The current state of understanding of these processes does not permit prediction of the success of either and does not provide the molecular biologist with guides for the construction of successful organisms. Study in these two fields was assigned the highest priority.

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The complete report, entitled "Genetic Engineering and the Development of New Pollution Control Technologies," (Order No. PB 84-148 972; Cost: $14.50, subject to change) will be available only from:
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Telephone: 703-487-4650
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