Chapter 11

U.S. Investment in Biotechnology Applied To Hazardous Waste Management

"The prospect of controlling pollution is one of the reliable rhetorical war-horses trotted out by advocates of the new biological technology every time someone asks what this new baby might be good for."

Douglas McCormick
Bio/Technology
May 1985

"If it wasn't for the high cost of the alternative, this (bioremediation) wouldn't be worth considering at all. "

Perry L. McCarty
Stanford University
July **1987**

"Burning and burying are no solution. They just make less of a bigger problem."

Ananda M. Chakrabarty
University of Illinois
September **1987**

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INTRODUCTION

Destroying persistent toxic waste is frequently touted as a major benefit of new biotechnologies. Natural microbial populations have a wide range of waste management capabilities, from degrading hydrocarbons to accumulating cadmium. While existing micro-organisms can degrade most natural chemicals, organisms frequently require some assistance to be effective against many man-made chemicals. Many applications of biotechnology for hazardous waste management are still experimental, and the investment in developing biotechnology for waste treatment and cleanup is small when compared with efforts in pharmaceuticals or agriculture. **Current applications rely on conventional techniques of genetic manipulation and microbiology; the use of recombinant DNA to develop microbes with special capabilities for waste degradation has been limited.**

Research and development in biological waste treatment methods is growing and may equal R&D efforts in thermal technologies. Companies using biological cleanup techniques have attracted substantial amounts of venture capital in recent years.

In this chapter, biotechnology for hazardous waste management refers to all efforts to engineer systems that use biological processes to degrade, detoxify or accumulate contaminants. These systems can use naturally occurring or laboratory-altered microbes or both. Genetic engineering refers specifically to the use of recombinant DNA techniques but does not include more conventional, less precise techniques of altering genes, such as random mutation and selection,

This chapter briefly describes the science underlying biotechnology for hazardous waste management and looks at some of the private and public sector activities in researching, developing, and applying new knowledge in biology to treat hazardous waste. The state of scientific knowledge and the barriers to further development of the field are analyzed.

This chapter focuses on issues specific to applying biotechnology to waste management, although some issues are generic to innovative waste treatment technologies. OTA has addressed many issues involved in waste management and waste reduction in its reports, ***Technologies and Management Strategies for Hazardous Waste Control* (93) *Protecting the Nation's Groundwater From Contamination* (90), *Superfund Strategy* (92), *Serious Reduction of Hazardous Waste* (91), *Ocean Incineration: Its Role in Managing Hazardous Waste* (89), *Wastes in Marine Environments* (94),** and ***From Pollution to Prevention: A Progress Report on Waste Reduction* (88).** Two related OTA studies are in progress: ***Municipal Solid Waste Management*** and ***Superfund Implementation.***

THE CONTEXT FOR RESEARCH

Several factors make the development of new technologies for waste management environmentally important and economically attractive. In 1985, U.S. industry generated at least 569 million metric tons of hazardous waste, according to EPA (103). Most hazardous waste has been put in unlined surface dumps, with no barrier between the waste and groundwater (54). The Federal Government has spent more than $2 billion on the cleanup of closed or abandoned waste sites, and industry has spent hundreds of millions more in complying with new Federal and State regulations on hazardous waste management (54). The Congress has strongly expressed its desire for hazardous waste generators to move away from land disposal and to use permanent treatment methods. These views are reflected in the Hazardous and Solid Waste Amendments (HSWA, public Law 98-616) of 1984 and the Superfund Amendments and Reauthorization Act (SARA, Public Law 99-499) of 1986.

Waste cleanup is a substantial and growing industry. The cost of waste disposal is expected to increase significantly in coming years. OTA has estimated that it will cost $300 billion over the next 50 years to clean up waste already generated (92). Gross annual costs of both solid and hazardous waste disposal have risen from $827 million in 1976 to $2.4 billion in 1984 (54). Arthur D. Little projected an $8 billion market for commercial hazardous waste treatment and disposal services by 1990, and the market could top $13 billion by 1995 (30).

Regulatory Pressures

Regulation both drives and constrains waste management practices. Within the last two decades the Federal Government has established regulatory and research programs to control and develop waste disposal activities. In addition to HSWA and SARA, the laws most pertinent to waste cleanup and disposal are the Toxic Substances Control Act (TSCA, Public Law 94-469), the Resources Conservation and Recovery Act (RCRA, Public Law 94-580), and the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA, Public Law 96-510).

In 1976, Congress passed the Toxic Substances Control Act to address comprehensively the risks of hazardous chemicals. The Act gives EPA highly flexible powers to control '(an unreasonable risk of injury to health of the environment," including the control of disposal methods (2).

Also in 1976, Congress passed the Resources Conservation and Recovery Act to cope with disposal of hazardous waste as it was generated. This program called for "cradle to grave" control of all hazardous waste and requires permits for treatment, storage, and disposal facilities.

RCRA was amended by the Hazardous and Solid Waste Amendments of 1984, which established deadlines for banning land disposal of many hazardous and persistent wastes. HSWA also required that all land disposal facilities monitor groundwater and certify financial responsibility by November of 1985. Fewer than one third of the 1,650 land disposal facilities certified compliance; the rest closed (53).

HSWA also greatly expanded EPA's authority to require corrective action for releases of hazardous wastes at RCRA facilities, where EPA has ultimate authority over what cleanup technologies are used. Therefore, if the agency develops the necessary knowledge base in biotechnology, it is possible that EPA would begin to recommend microbial degradation for RCRA corrective actions **(109).**

In 1980, Congress responded to rising public concern about hazardous waste sites with the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA or "Superfund"). Superfund requires the generator, transporter, and disposer of waste to bear the burden of cleaning up existing nonconforming disposal sites. The EPA has responsibility for monitoring and implementing cleanup at these sites. Superfund was originally funded for 5 years at $1.6 billion. The law was reauthorized in 1986 at $8.5 billion by the Superfund Amendments and Reauthorization Act (SARA, public Law 99-499).

Among the important provisions of the new law are deadlines for initiating cleanup actions; cleanup standards that emphasize permanent remedies; a program to accelerate cleanup at Federally owned hazardous waste sites; and broad new research and development authorities (73). In authorizing SARA, Congress mandated that the President shall "utilize permanent solutions and alternative treatment technologies or resource recovery technologies to the maximum extent practicable" (Public Law 99-499).

Thus, the regulatory environment is increasing pressure on waste generators to reduce waste and to find permanent solutions to the waste that is generated. over the past 2 years, regulations have banned the land disposal of solvents and other wastes. The Land Disposal Restrictions of HSWA stipulate that, by 1990, all RCRA hazardous wastes must meet certain treatment standards before they can be land disposed. Small-quantity waste generators, previously exempt, must now comply with regulations. In addition, SARA directs EPA to choose permanent remedies when possible, rather than burying wastes.

Economic Pressures

Regulations have and will continue to increase the cost of waste disposal, making alternative technologies more economically feasible. EPA reported that design and construction standards for RCRA-approved landfills raised the price of land disposal from as little as $10 to $15 per metric ton in the early 1970s to $240 per metric ton in 1986 (98). According to another report, prices charged by commercial waste management firms increased 30 to 400 percent in 1985 alone (99).

These price increases, moreover, predate the enactment of most provisions of the Hazardous and Solid Waste Amendments of 1984 (HSWA). HSWA requirements have already resulted in the closure of some 1,100 noncompliant land disposal facilities. If implemented as enacted, HSWA will force most land disposal facilities to install liners and leachate collection systems and will prohibit land disposal of wastes for which alternative treatment methods exist (54).

EPA estimates that the HSWA will add at least $2.25 billion to industry's annual cost of waste disposal, approximately doubling 1984 disposal costs (54), although this estimate does not reflect potential savings from waste reduction and lower-cost on-site disposal.

Companies with new technologies and services for waste management are seeing sales increase at 20 to 30 percent per year (52). Stock prices of six waste companies followed by Kidder Peabody & Co. rose substantially higher than Standard and Poor's 500-stock" index from 1984 until the October 1987 stock market crash and have rebounded strongly from the crash (46).

SCIENTIFIC BASE OF BIOTECHNOLOGY FOR WASTE MANAGEMENT

The rationale for using micro-organisms to degrade pollutants comes from experience with nature. Micro-organisms, particularly bacteria, have a variety of capabilities that can be exploited for waste management and disposal and have been intentionally used for municipal waste management for over a century.

A large proportion of organic compounds of biological and chemical origin are biodegraded, predominantly by micro-organisms (69). Organic compounds of biological origin are readily degraded. Many different micro-organisms are known to degrade oil (19). Industrial chemicals that are similar in structure to natural compounds are frequently also biodegraded.

Persistent Chemicals

Persistent compounds, however, have chemical structures not found in natural compounds and so resist degradation by most naturally occurring micro-organisms. Such compounds are called ***xenobiotics.*** In addition to xenobiotics, other compounds may persist in the environment, because the compounds are present in too dispersed or too toxic a concentration, the organisms necessary for degradation are absent or occur in low amounts, one organism cannot degrade the compounds completely, or the oxygen and nutrients necessary for degradation are lacking.

Industrial chemicals have been present in the environment for "only an instant in evolutionary time" (69), a period that is often not long enough for the evolution of the necessary catabolic enzymes, the molecules made by organisms to bring about degradative reactions. Micro-organisms, however, display "a striking plasticity" to evolve the necessary capabilities and, on occasion, to do so in a short amount of time (83) and sometimes evolve new pathways rapidly when confronted

Table 11-1.—State of Knowledge of Biodegradation of Common Pollutants[a]

Pollutant	Organism degrades or transforms	Pathway known	Enzymes characterized	Genes sequenced	RecDNA technology	On-site biodegradation underway
Acetone	⊕	⊖	⊙	⊙	⊙	⊙
Aluminum and Compounds	⊙	⊙	⊙	⊙	⊙	⊙
Anthracene	⊕	⊕	⊖	⊙	⊙	⊕
Arsenic	⊕	⊕	⊙	⊙	⊙	⊙
Barium	⊙	⊙	⊙	⊙	⊙	⊙
Benzene	⊕	⊕	⊕	⊖	⊖	⊕
Benzo(a)Pyrene	⊕	⊖	⊙	⊙	⊕	⊕
Bis(2-Ethylbenzyl) Phthalate	⊕	⊖	⊙	⊙	⊙	⊙
Cadmium (Cd)...	⊖	⊕	⊕	⊙	⊕	⊙
Carbon Tetrachloride	⊕	⊖	⊖	⊙	⊙	⊙
Chlordane.	⊖	⊖	⊖	⊙	⊕	⊙
Chlorobenzene	⊕	⊕	⊖	⊖	⊖	⊙
Chloroform	⊕	⊕	⊙	⊙	⊙	⊙
Chromium	⊖	⊙	⊖	⊖	⊖	⊙
Chromium, Hexavalent.	⊕	⊖	⊙	⊙	⊙	⊙
Copper and Compounds (Cub)	⊙	⊙	⊙	⊙	⊙	⊙
Cyanides (soluble salts).	⊕	⊕	⊖	⊙	⊙	⊕
DDT.	⊕	⊕	⊖	⊙	⊕	⊙
Dichloroethane	⊕	⊖	⊙	⊙	⊕	⊙
1,1-Dichloroethane	⊕	⊖	⊙	⊙	⊙	⊙
1,2-Dichloroethane	⊕	⊖	⊙	⊙	⊙	⊙
1,1-Dichloroethene	⊕	⊖	⊙	⊙	⊙	⊙
Ethylbenzene	⊕	⊕	⊖	⊖	⊙	⊙
iron and Compounds	⊕	⊙	⊙	⊙	⊙	⊙
Lead (Pb)	⊖	⊖	⊙	⊙	⊙	⊙
Lindane.	⊕	⊖	⊙	⊙	⊕	⊙
Manganese and Compounds (Mn)	⊕	⊙	⊙	⊙	⊙	⊙
Mercury	⊕	⊕	⊕	⊕	⊕	⊕
Methyl Ethyl Ketone	⊕	⊖	⊙	⊙	⊙	⊙
Methylene Chloride	⊕	⊕	⊙	⊙	⊙	⊙
Naphthalene	⊕	⊕	⊖	⊖	⊕	⊕
Nickel and Compounds (Ni)	⊕	⊖	⊖	⊙	⊕	⊙
Pentachlorophenol (PAP).	⊕	⊕	⊖	⊙	⊕	⊕
Phenanthrene	⊕	⊕	⊖	⊖	⊙	⊕
Phenol	⊕	⊕	⊖	⊖	⊙	⊕
Polychlorinated Biphenyls (PUBS)	⊕	⊕	⊖	⊖	⊖	⊕
Pyrene.	⊕	⊖	⊙	⊙	⊕	⊕
Selenium	⊕	⊙	⊙	⊙	⊙	⊕
1,1,2,2-Tetrachloroethane	⊕	⊕	⊙	⊙	⊙	⊙
1,1,2,2-Tetrachloroethene.	⊕	⊕	⊙	⊙	⊕	⊙
Toluene.	⊕	⊕	⊕	⊖	⊖	⊕
1,2-Trans-Dichloroethylene	⊕	⊖	⊙	⊙	⊙	⊙
1,1,1-Trichloroethane	⊕	⊕	⊕	⊕	⊕	⊙
1,1,2-Trichloroethane	⊕	⊖	⊙	⊙	⊙	⊙
Trichloroethylene (TEE)	⊕	⊖	⊖	⊙	⊕	⊕
Vinyl chloride	⊕	⊖	⊙	⊙	⊙	⊙
Waste Oils/Sludges	⊕	⊙	⊙	⊙	⊙	⊕
Xylenes	⊕	⊕	⊙	⊙	⊖	⊕
Zinc and Compounds (Zen)	⊖	⊙	⊖	⊖	⊖	⊙

KEY: ⊕ - Known
e - Partially Known
⊙ - Not Known

[a]This table was compiled from information provided toOTA by 20 researchers in the field of biodegradation?. Some compounds listed include multiple congenors, for which organisms may be known that degrade some congenors but not others.

SOURCE: Office of Technology Assessment, 1988.

with significant concentrations of xenobiotics in their environment.

Micro-organisms have been identified that degrade at least 42 pollutants commonly found at hazardous waste sites targeted for cleanup on the National Priority List (NPL) sites (table 11-1). Current research is aimed at exploiting the natural degradative capabilities of microbes to accelerate degradation, enable the organisms to live in new environments, and attack new contaminants.

Simple Bioremediation

Waste management biotechnology typically involves mixing live organisms or their products with the waste to degrade or transform it. Biological treatment requires that an organism live in a sometimes exceedingly hostile environment. In nature, certain organisms live in extreme environments, Thus, in some cases, it has been possible to isolate micro-organisms from a particular environment (where they have been environmentally selected) and introduce them into similarly contaminated sites. Alternatively, supplying the required nutrients and conditions may allow organisms already present to degrade waste. Isolated organisms may also be further adapted in the laboratory with mutagenizing agents (e.g., radiation) or with selective pressure (see figure 1). Many bacteria have such short generation times that under strong selective pressure a year is more than enough time to evolve desired characteristics. Under the right laboratory conditions, many bacteria can divide about every two hours (or faster), creating over 4,000 generations per year. Such a large number of generations provides significant opportunity for evolution (51).

In these cases, almost nothing may be known about the biochemistry or the genetics of the organism that breaks down the pollutant. Lacking comprehensive biodegradation and physiological information, strategies to enhance degradation involve reseeding the site with bacteria as they die out (bioaugmentation) or enhancing the site with nutrients or oxygen required by the micro-organisms for optimum growth and performance (bioenrichment). For simple waste sites involving readily degraded contaminants, such as fuel oils, these strategies suffice.

Remediation of Complex Sites

Waste sites pose significant challenges to organisms. Waste sites can involve materials in any form: solid, liquid, gas, or mixed. Waste sites can involve a single material, a family of related compounds called congeners, or a mix of unrelated wastes. Pollutants in lagoons or landfills may leach into the groundwater. Pollutants may occur highly diluted, highly concentrated, or in locally concentrated "hot spots" (13).

Different environments require different strategies. Immobilized enzymes in a bioreactor may be the best method to treat a waste stream at the source. As complexity grows, a single organism may not be able to survive or compete in the contaminated environment. To clean up an ecosystem, an ecosystem-level approach may be required, incorporating a variety of organisms (49) (see figure 11-2).

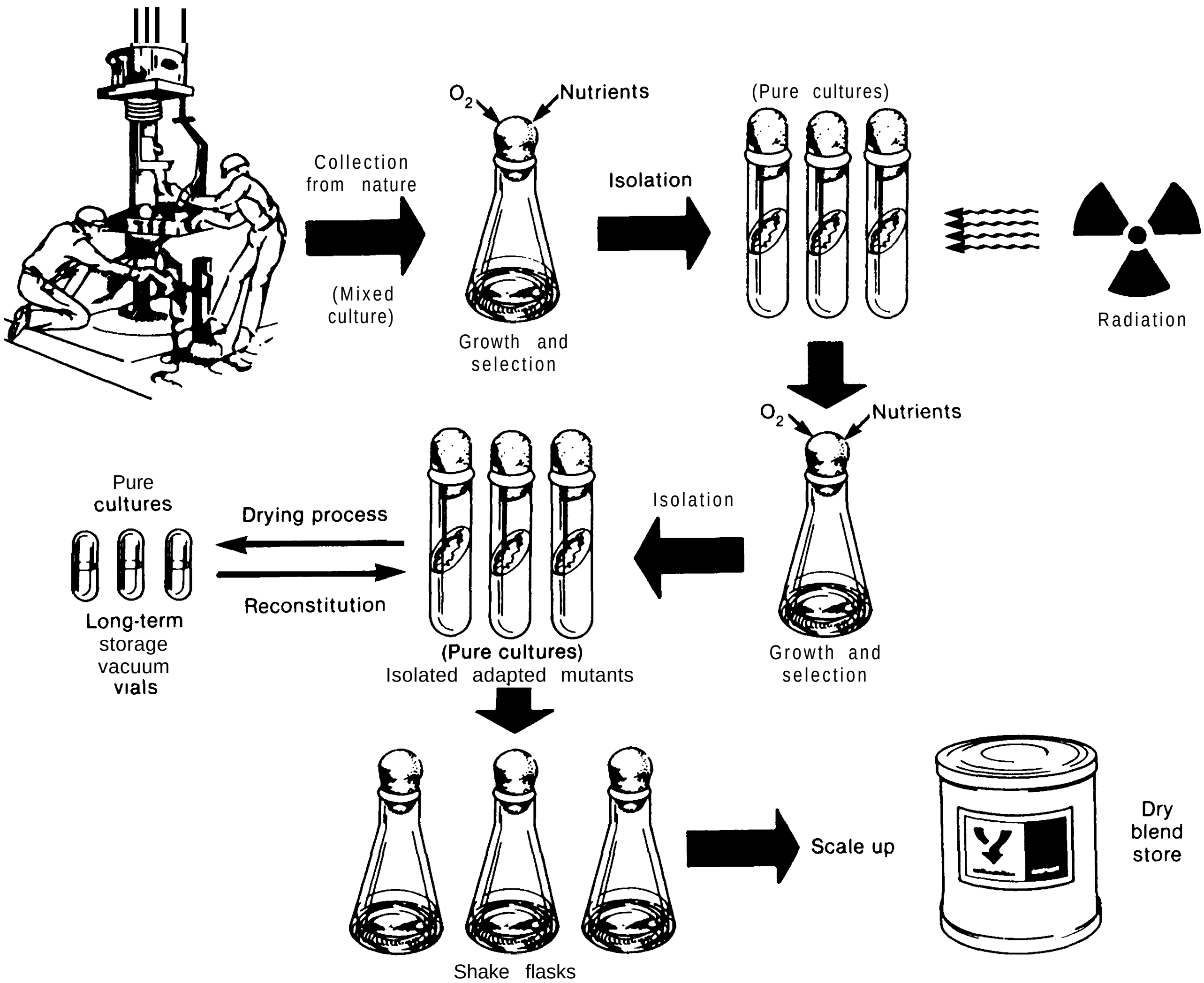
Environmental conditions affect organism function. Although an organism can degrade or otherwise change a toxic chemical, it might do so only at certain concentrations or at a relatively slow rate. Mixtures of chemicals at sites might poison the organism, or the degradation reaction might supplant other necessary reactions, such as energy production. Many organisms require oxygen, which might not be available in the site. Finally, many pollutants occur attached to particles or in other physical states that can make them unavailable to the organism (76).

Finally, degradation itself may be the limiting factor in the use of biological systems for site remediation. If the waste provides the sole carbon source for the organism, then as the waste is depleted, the food supply for the organism is also depleted. The organism may or may not survive as the waste reaches lower concentrations. Thus, full remediation may not be achieved.

Modern Biological Strategies

The term degradation generally indicates that a product is changed, but not necessarily the extent to which it is altered or broken down. Many demonstrations of degradation rely on evidence that a single compound is lost, without determining whether new products are formed (76). Degra-

Figure 11-1.—Laboratory Selection and Enhancement of Micro-organisms



Micro-organisms indigenous to various environmental sites can be isolated and screened for degradative capabilities. This figure shows how naturally occurring organisms can be selected in the laboratory and, if desired, subjected to mutagenizing agents such as radiation. This imprecise method can sometimes produce new strains of organisms with enhanced capabilities.

SOURCE: Polybac Corp.

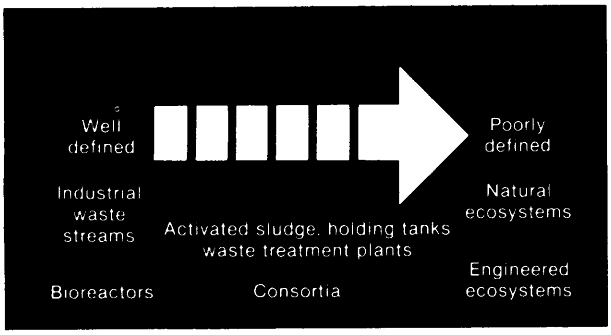
dation can produce new toxic products (76). Simply defined, true biodegradation is the metabolism of a compound to innocuous products (35). It is therefore necessary to identify the pathways of degradation and define the acceptable products and amounts.

With better knowledge of microbial genetics, microbial physiology, and microbial ecology, scientists and engineers can develop more efficient strategies for biodegradation.

Ideally, a complete biological strategy for research and development to degrade a pollutant would include:

- finding and characterizing an appropriate organism with degradative capabilities;
- defining the conditions that allow the micro-organism to exist and function;
- defining the pathway of metabolism for the pollutant and for any other related or critical cell products;

Figure 11-2.—The Continuum of Environments in Xenobiotic Degradation



THE CONTINUUM OF ENVIRONMENTS IN XENOBIOTIC DEGRADATION: A wide variety of environments means that in order to eliminate toxic materials a variety of strategies must be employed. In waste streams from process plants an immobilized enzyme in a bioreactor may prove sufficient. As complexity grows a single organism may not be able to survive or compete in the contaminated environment. In order to clean up ecosystems an ecosystem level approach may have to be undertaken, incorporating a variety of organisms and trophic levels.

SOURCE" Wayne G. Landis, Chemical Research, Development and Engineering Center, Aberdeen Proving Grounds, MD.

- identifying and characterizing the enzymes of the pathways; and
- characterizing the treatment environment.

If genetic engineering is applied, these additional steps are necessary:

- locating the genes for the enzymes and the control of the pathways; and
- manipulating the genes to improve degradation rates, stability, or substrate range.

In some applications, sequencing the genes of interest may provide some clues for ways to alter gene products to degrade persistent compounds.

Metabolic Pathway Design

Three approaches are being used in the laboratory to design beneficial metabolic pathways (the first two are more commonly used):

- chemostats and other laboratory systems, in which organisms are grown under long-term selective conditions to encourage the organisms to metabolize new substrates;
- in vivo ***genetic transfers,*** in which the gene of a useful enzyme from one organism is recruited into a pathway of another organism via natural genetic processes; and
- ***recombinant DNA technology,*** in which genes are introduced by in vitro techniques into a new host to create a new pathway.

Recombinant DNA technology enables the most precise manipulation of genes, but also requires extensive background knowledge and thus research and development. Selective pressure and in vivo transfer can often be accomplished without extensive basic research. In certain cases, the waste site itself has provided selective pressure to generate organisms capable of metabolizing new substrates, such as the decades of exposure to creosote and pentachlorophenol (PCP) waste.

EPA and University of Illinois scientists have used the in vivo transfer strategy to further modify a strain of ***pseudomonas*** isolated from a chemostat. The transformed ***Pseudomonas*** can completely degrade 2,4,5-T, one of the active ingredients of Agent Orange. This strain carries a plasmid (an extrachromosomal unit of DNA) with the genes responsible for making one or more enzymes that degrade the compound. Modifying the plasmid so that it can be introduced and maintained in a range of host organisms that can exist in toxic sites could lead to environmental application (37).

Genetic Enhancement of Organisms

One strategy uses recombinant DNA technology to rationally design pathways that can degrade xenobiotic compounds. These pathways can be constructed in two ways: restructuring existing pathways or assembling entirely new pathways from enzymes or portions of enzymes (37). The latter strategy is called patchwork assembly.

Many perceive the benefits of using, wherever possible, natural pathways in indigenous organisms while using recombinant DNA technology to develop reactions for recalcitrant compounds. Work is progressing on molecular biological approaches to several classes of recalcitrant compounds. For example, a pathway is known that degrades DDT, one of the most persistent pesticides in the environment, to DCB, an acceptable product (76). However, one step in the pathway requires oxygen. Eliminating the oxygen requirement would be advantageous in many applications. Basic research in recombinant technology is needed to develop organisms that will work without oxygen (34,58,61,81).

Other laboratories are working on genetic approaches to facilitate the removal of toxic forms of metals, which pollute various waste streams and soils. This process can reclaim valuable metals (12,41,84,108).

Useful Microbial Properties

In some cases, the pathways, enzymes, and genes are known and available to degrade a pollutant, but the conditions in which the pollutant exists inhibit or kill the organisms. Such conditions include unusual concentrations of the pollutant, extreme temperatures, high salt concentrations, extreme pH, and the presence of additional chemicals that are toxic to the organism (76). Genetic approaches to these problems include increasing the activity of a gene so the organism can live in more toxic concentrations or placing the requisite genes in organisms that can exist in these extreme environments.

Linking genes for surfactants and emulsifiers with genes for degradation may permit organisms to work more effectively (21), since the physical state of the pollutant is often critical to degradation. pollutants frequently occur in partially solid lagoons where the chemical adheres to soil (55, 76) or is mixed with oil.

The search for degradative activity has turned up two reactions with surprising and potentially broad applications. In one, the enzyme ligninase degrades lignin, a naturally occurring compound that resists degradation by most microorganisms. The enzyme has been reported to partially degrade PCBs, dioxin, lindane, PCP, and DDT (11, 15,116). For practical applications, low concentration of the pollutant is a problem as the enzyme may attack other materials in the site rather than the target pollutant.

In the other a newly discovered anaerobic reaction breaks the chlorine-carbon bond in aromatic compounds-one of the most recalcitrant chemical bonds and a major stumbling block in the destruction of wastes. Removing the chlorine is a key step in degrading PCBs, chlorinated benzenes, chlorinated phenols, and dioxins (76). This recently discovered anaerobe, isolated from sewage sludge, removes the chlorine from chlorobenzoate, producing benzoate. While chlorobenzoate is not a major pollutant, it serves as a model for major pollutants (29). Recently, this dechlorination reaction has been shown to work on hexachlorobenzenes and some PCBs (33).

Microbial Physiology and Ecology

Research and development in microbial physiology and ecology are much less developed than microbial biochemistry and genetics. These aspects have serious implications for the use of organisms in the environment to reduce waste and pollution.[1] Only 1 to 10 percent of all soil organisms are known or cultured (22). Even for known microbes, little is known about the entire set of reactions that occur in any one organism or how these reactions are interrelated and controlled. Even less is known about the relationship of an organism to its environment and to other organisms. Knowledge of physiology and ecology is especially important in nutrient enrichment and bioaugmentation. otherwise, the efficiency and the outcome of the biosystem cannot be known.

Microbial Communities

Micro-organisms are not isolated in the environment but occur in mixed microbial communities. Microbial communities are sometimes able to degrade pollutants that a single organism could not. If the conditions are right, a series of reactions can be accomplished by the community of organisms. For example, the dechlorination reaction described previously is followed by at least two other reactions carried out by other specialist organisms, one that transforms benzoate into acetate, hydrogen, and carbon dioxide, and another that converts hydrogen and carbon dioxide into methane (29).

In another case, enrichment by an analog chemical, a chemical similar in structure to the pollutant but without the chlorine attached, causes the

[1] A more thorough discussion of microbial ecology and other aspects of the environmental application of novel organisms can be found in OTA's report, *Field-Testing Engineered Organisms: Genetic and Ecological Issues (87).*

requisite bacteria to grow and induces degradation, Then other organisms, which have not yet been isolated, metabolize the products. Creating new genotypes that would work in concert with the indigenous organisms could replace the analog chemical. At least one laboratory is exploring this strategy (34). Providing oxygen and nutrients increases the cost of treatment substantially, both in the cost of the raw material and in the cost of supplying and mixing in the additives. Reducing the need for additives would produce significant savings.

Exploring Other Organisms

One approach to reducing the need for additives is to use anaerobes, which do not require oxygen. Anaerobes might also be capable of novel reactions. Basic knowledge of most anaerobes is lacking, and genetic engineering of anaerobes is problematic. Researchers are, however, beginning to systematically seek reactions in anaerobes that can be developed to efficiently degrade aromatic hydrocarbons in soil (34,47,58).

While most research in the past has focused on bacteria, other organisms also perform desired reactions, such as fungi (15,38), algae (74,88), protozoa (50), yeast (107), clams (50), and plants (7,4 I).

Site Engineering

Biotechnology waste treatment sites provide significant and unique challenges to environmental engineers. The site engineer must ensure that the organism degrades the pollutant, treating the contaminant directly where it occurs (in situ), using a contained bioreactor, or using a combination of these two. Nonbiological technologies, such as air stripping (the removal of volatile compounds via a jetstream) and incineration, maybe used in conjunction with biological treatment.

Site engineers must consider at least five criteria:

- The availability of the contaminants. Getting microorganisms into contact with sorbed or nonaqueous contaminants is often limiting for oils, some solvents, pesticides, dioxins, and PCBs (74).
- The ability of the degrading strains to live and function. The ecology of the treatment environment determines whether or not desired micro-organisms will survive and do their job (74).
- The ability to degrade pollutants at very low concentrations. Achieving the very low concentrations usually required for hazardous waste cleanup requires micro-organisms adapted to function at low concentrations or reactor conditions designed to allow very low concentrations (74).
- The ability to cope with a range of conditions, particularly unexpected substances and concentrations.
- The need for low costs. Cost-effective approaches to waste cleanup require designs to minimize costs of equipment, energy, and manpower. The cost of the organisms, once identified and developed, is relatively low. Cost-effectiveness requires reactor designs that minimize initial capital and operations costs (74).

Demonstration projects have shown that appropriate bacteria will metabolize pollutants in landfills if they are maintained with proper energy sources and nutrients in thin layers of well-prepared, hydrated soil. For soil pollutants, a technique called landfarming may be used (14,60,85). This involves establishing treatment domains, pretreating the soil for pH and other conditions, spraying the soil with the micro-organisms, and maintaining the site with the proper humidity, oxygen, and nutrients. Providing nutrients and oxygen and mixing the components properly are major tasks. In current applications, treatment has been confined to surface layers (1.5 to 6 feet) of soil (44,72,85).

Currently data concerning the optimal ratios of microorganisms and nutrients to pollutants are largely derived from laboratory trials. Scale-up on site is difficult (76). Field conditions can vary significantly, changing expected results.

Most waste sites present a variety of problems for in situ treatment. Frequently wastes are ***mixed*** and in extremely varied concentrations, making both assessment and treatment difficult (8,76). In lagoons, liquid pollutants may adhere to solids, significantly reducing reaction rates (55,56). La-

Photo credit: Ecova Corp.

Daily tilling of soil provides oxygen to naturally occurring microbes, enabling them to remediate hydrocarbon-contaminated soil in an enclosed, solid-phase soil treatment facility. Current applications of biotechnology to waste management rely on naturally occurring microbes; the application of genetic engineering to this field remains some years away.

goons frequently leak and the pollutants may be found in the unsaturated (vadose) zone or in the water-saturated (aquifer) zone, contaminating the groundwater.

Among the most difficult sites to clean up is groundwater that contains low molecular weight, semi-volatile substances, such as trichloroethene (TCE), a widely used industrial solvent. In these cases, in situ treatment means injecting materials to create a reaction site in the groundwater. Thus, the resulting products and the migration of contaminants must be well understood. In situ treatment of soil contamination, on the other hand, can increase groundwater contamination, at least temporarily, as contaminants are released from the soil. Then the degradation reaction can occur in the groundwater (65).

In order to better control the conditions of the reactions and circumvent some of the safety concerns related to in situ treatment, bioremediation companies often use contained bioreactors. Here the process is more analogous to fermentation or sewage treatment technologies, where the pollutant is passed through a closed or controlled system. Considerable research is underway to optimize such bioreactor systems. Promising techniques include the use of fixed films (10,67), fluidized beds (75), microbes immobilized on beads (25,36,62), and microbes immobilized by membranes (4).

Directly at the end of a waste stream, the pollutants may be known, consistent, relatively pure, and moderately concentrated. Because of these factors, there is emerging interest in the use of biotechnology to treat an undesired prod-

uct within the waste stream or directly at the end of the pipeline where the cost of collecting the material is less and the conditions may be better controlled (18,36,91,110,1 11). In some cases, biotechnology might be used for waste reduction. For example, the use of ligninase to pulp wood could reduce the air and water pollution of chemical pulping (42).

BIOTECHNOLOGY APPLICATIONS IN HAZARDOUS WASTE MANAGEMENT

The application of biotechnology to hazardous waste management is new and less developed than applications in the pharmaceutical or agricultural industries, The relative merits of conventional versus biotechnological approaches to waste management are currently being debated. **Questions regarding the effectiveness and economic attractiveness of biotechnological techniques for waste management have not been resolved.** Conventional methods, e.g., airstripping, incineration, and containment, have a longer history, are better understood, and are thus frequently preferred, Company representatives have reported difficulty obtaining permits for biological remediation techniques (26,86). Such difficulties are common for innovative treatment technologies.

In contrast, certain industries historically have used conventional biotechnology to use or treat waste, have matter-of-factly adopted limited innovations in the field, and are convinced of its economic advantage. In other cases, changes in the regulatory environment (such as California's recent ban on open airstripping of volatile), poor economic projections of the conventional technologies, or reduced availability of dump sites have forced some industries to explore new technologies.

At least 65 companies are involved in some aspect of biotechnology for waste management (see app. D). Some of these companies are dedicated biotechnology companies (DBCs, see ch. 5), others are waste management companies, and some are waste generators. A few companies have fully functioning sites relying on micro-organisms or products of micro-organisms to detoxify waste. Other organizations are engaged in demonstration projects. Several independent and young companies dedicated to biotechnological waste treatment have emerged.

No waste management company is currently using or even testing genetically engineered micro-organisms in the environment, although research on genetic engineering of model organisms is proceeding in laboratories. Current, on-site biotechnology strategies in the waste industry involve the use of either environmentally selected organisms or laboratory adapted, crossed, or mutagenized strains. Biological approaches are frequently integrated with conventional approaches.

Current applications of biological degradation focus on fuel oils, common industrial solvents such as benzene, wood preservatives such as PCP and creosote, and other compounds that are relatively amenable to biodegradation. One company president expressed a commonly held feeling in saying that there is so much crude oil, benzene, and diesel oil spilled around the country that there is no need to look for more exotic applications (115).

Various cost savings are attributed to biodegradation systems over other methods, but generalizations are difficult to make due to the variability of waste sites. **While various claims of cost savings have been made, few if any demonstrations by disinterested parties, such as EPA or state environmental agencies, clearly evaluate the cost and effectiveness of biological cleanup compared with other cleanup technologies.**

Waste Stream Cleanup

Bethlehem Steel Company uses a conventional biological approach to handle the coke oven waste water at its Sparrows Point, MD, plant. The coke oven waste stream contains phenols, cyanides, and ammonia, comprising about 4,000 to 6,000 pounds per day of phenol. Prior to 1970, the waste was dumped directly into the Chesapeake Bay. Now the waste stream goes through the equivalent of a sewage treatment facility. This facility is seeded with sludge from local sewage that contains naturally acclimated microbes. The daily output of phenol is reduced to about 2 pounds (99.9 percent reduction), a level in compliance with the National Pollution Discharge Elimination System for discharge of water.

The Sparrows Point plant was used to set EPA's "Best Available Technology" standard. Economic evaluations from the 1970's showed that the biological treatment plant was less expensive to build than other methods at that time by $1.2 million and is simple and inexpensive to run ($1.7 million as compared with $2.6 million for the conventional treatment for one year in 1978). All but one other steel plant in the United States have adopted this treatment method, although recent changes in effluent standards, requiring treatment of ammonia, are again forcing a change in treatment technology. Bethlehem Steel had been examining the use of biological methods to degrade the petroleum hydrocarbons in steel rolling mill solid waste at a site in Bethlehem, PA, until poor economic conditions forced the company to cut back at all levels and terminate its program on biotechnology (80).

Wood Treatment Site Cleanup

Wood preservation plants have created a significant number of waste sites. Koppers Company, a diversified manufacturing company with interests in wood preservation plants, has created a subsidiary environmental services company, Keystone Environmental Resources, Inc., to deal with waste sites resulting from wood preservation plants. Keystone received EPA funds for a demonstration project to clean up a wood preservation site in Nashua, NH, that contained creosote, polynuclear aromatic hydrocarbons (PAHs), PCP, and dioxins in soil. The treatment system was established on a prepared bed of soil and loaded with a 1-foot layer of soil augmented with cow manure and fertilizer. The soil was sprayed periodically with water and tilled once a week to improve mixing and aeration (44). In 5 months of degradation, over 75 percent of PCP and over 95 percent of polynuclear aromatic hydrocarbons (PAHs) were degraded. Neither the groundwater nor the soil beneath the system were affected by the chemicals in the treatment system (86). The soil went from being visibly contaminated with oil and grease to the consistency of garden soil "which might be used for construction site fill" (44).

PCB Degradation

General Electric Corp. (GE) has extensive contamination problems resulting from the widespread use of polychlorinated biphenyls (PCBs) in electrical transformers beginning about 50 years ago (13). GE began examining the use of micro-organisms to biodegrade PCBs and other contaminants in 1981. In the laboratory, they isolated 35 to 40 mixed cultures; upon purification of two dozen of these cultures, several strains were found to degrade PCBs exceptionally well, and two of these showed novel pathways. The genes for PCB-degrading enzymes from one strain have been cloned. The first laboratory demonstration project treated soil spiked with PCBs; next, soil from a contaminated site was treated in the laboratory. As of September 1987, a site test was underway at South Glens Falls, NY, where oil containing PCB was used for dust control on a race track. GE provides its strains to other companies and academic laboratories. (85). None of these strains, however, degrades the more persistent highly chlorinated PCBs (82).

Chemical Manufacturing Wastes

The Occidental Chemical Corp. is responsible for a number of chemical dump sites, including Love Canal. Occidental, its subsidiary TreatTek, and BioTal (formerly BioTechnica Ltd.) have claimed full-scale remediation assisted by microbial technology at two sites.

The Hyde Park Landfill in Niagara, NY, was used from 1963 to 1975 as a disposal site for an estimated 73,000 metric tons of chemical waste, including phenols, halogenated organics, and halogenated aromatic compounds (especially chlorinated benzoic acids), including dioxin. A compacted clay cover was placed over the landfill in 1978, and a leachate collection system made of tile was installed around the perimeter in 1979. The leachate is collected in a sump, pumped into a lagoon, the lagoon allowed to settle, and the supernatant trucked to a nearby treatment plant. The conventional treatment uses activated carbon, at an estimated cost of $21 million over the next 10 years. The company developed batch bioreactors using organisms selected from contaminated sites, which reduce the need for activated carbon by 96 percent, saving an estimated $20 million at this site alone.

At an abandoned gasworks site in England, coal tars, phenols, cyanides, heavy metals, and other contaminants were similarly treated by traditional methods, supplemented by microbial methods, to reduce phenols from 500 to less than 100 mg/kg in 8 weeks. Full-scale treatment will require excavation to layer the soil (112,113)114).

Groundwater Treatment

At a Superfund site in California, Ecova Corp. operates a groundwater decontamination system that combines an air stripper with a bioreactor to remove chlorinated hydrocarbons and soluble organics. The air stripper is a 35-foot column that blows air at a cascade of groundwater, thus stripping volatile hydrocarbon molecules from the water. After removal of volatile organics, the groundwater is transferred to a bioreactor to degrade the soluble organics. The bioreactor is a 10,0()()-gallon tank seeded with microbes and a nutrient mix developed specifically to biodegrade the remaining soluble organic contaminants. The bioreactor contains an agitator to provide aeration and instrumentation to monitor contaminant levels and rates of degradation. The treated groundwater meets standards established by the California Regional Water Quality Control Board (chlorinated hydrocarbons of 5 ppb and soluble organics of 1 ppm), and the effluent can be discharged to the public sewer system (32).

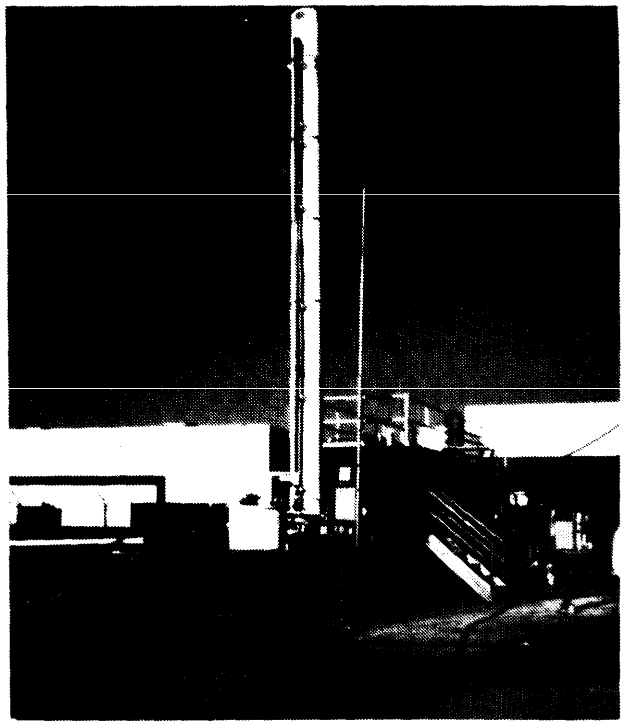


Photo credit: Ecova Corp

An air stripper, combined with a bioreactor, detoxifies wastes at this Superfund site in San Jose, CA. The air stripper blows air at a cascade of groundwater to remove volatile hydrocarbons. The groundwater is then transferred to the bioreactor where soluble organics are degraded.

RESEARCH AND DEVELOPMENT FUNDING

Research and development funding for biotechnological approaches to waste management is modest compared with funding in other areas of biotechnology and comes from a variety of public and private sources. In addition to basic research, which has the potential for leading to innovations in all fields of biotechnology (supported by projects in genetics, molecular biology, microbial physiology, and ecology), the waste biotechnology field is supported by basic research concerned with toxic compounds, environmental sciences, physical-chemical sciences, and engineering,

Public Sector Investment

A substantial portion of basic research underlying biotechnology for waste management is supported by the public sector through the regular

Table n-2.-Federal Expenditures for Biotechnology Applications to Waste Cleanup

Agency	Fiscal 1987 Dollars (in thousands)
Department of Defense	1,953
Department of Education	486
Department of Energy	920
Department of Interior	714
Environmental Protection Agency	3,497
National Aeronautics and Space Administration	350
National Institutes of Health	270
National Science Foundation	2,750
TOTAL	10,942

SOURCE: Off Ice of Technology Assessment, 1988.

intramural and extramural programs of the Federal agencies. Federal agency funds specific to biotechnology for waste control are listed in table 11-2, for fiscal year 1987. EPA invested the most of any Federal agency, spending about $3.5 million on R&D related to biological systems for waste management. This is less than one-third of the total Federal investment, which OTA estimates at almost $11 million (table 11-2).

EPA Activities

The Environmental Protection Agency is the principal agency for conducting research and development for biotechnology and waste disposal. However, EPA is primarily a regulatory agency, and most of its R&D is geared to support regulatory activities. Thus, most biotechnology funds are directed toward developing methods for risk assessment. EPA also has some funds for developing products to clean up waste or products to mitigate risks of environmental damage. Funding levels for product development research are low, however, in accordance with EPA policy that the private sector should play a primary role in the development of products for commercial use (48).

Nonetheless, EPA laboratories are conducting a variety of small but significant research projects (see table 11-3). EPA also sponsors documentation and evaluation of new cleanup technologies through the Superfund Innovative Technology Evaluation (SITE) Program, and a coordinated Biosystems Initiative is planned for fiscal year 1989, pending budgetary approval. Many EPA projects involve innovative biological treatment technologies, but many do not involve genetic engineering, and so fall outside of EPA's definition of biotechnology (see ch. 3). Thus, funding figures reported in this chapter overlap with EPA funds reported in chapter 3 only for projects that involve genetic engineering of organisms for waste degradation,

SITE Program. The Superfund Innovative Testing and Evaluation (SITE) Program is authorized under the Superfund Amendments and Reauthorization Act of 1986. The SITE Program provides testing, sampling, and evaluation of innovative technology for hazardous waste cleanup. The proprietor of the technology pays for the demonstration itself, if private funding is available. If funding is not available, EPA can fund up to 50 percent, not to exceed $3 million, for any single demonstration. EPA accepted 12 technologies for testing and evaluation in the first round of selections for the SITE program in April 1987. One of these involves the microbial degradation of PCBs. In September of 1987, EPA selected 3 biologically based technologies out of a total of 10 for the second round of selections (39,45).

Funds have not yet been spent by EPA for the PCB-degradation project, but preliminary estimates indicate that testing and evaluation will cost about $200)000 (45), The owner of the technology says the SITE demonstration will cost him $50,000 and 1 year's time. He says that this demonstration will involve the bioremediation of 10 cubic yards of soil, although he has already demonstrated the technology on 14,000 cubic yards under State auspices. The SITE program, however, will provide the documentation and analysis to assure potential clients that the system works (26).

Biosystems Initiative. Recognizing the potential of biological systems for waste management, EPA proposed the Biosystems for Pollution Control Initiative, which, if approved, would begin in fiscal year 1989, The proposed initiative would provide about $4 million per year from 1989 to 1991 to develop, demonstrate, and evaluate biological technologies for waste cleanup (39). The Biosystems Initiative includes the following objectives:

- search out and characterize biodegradation processes in surface waters, sediments, soils,

Table 11-3.—EPA Projects in Biotechnology for Waste Management[a]

Project	Dollars (fiscal 1987)
Environmental Research Laboratory, Gulf Breeze, Florida	
TCE degradation	
Complex waste sites	
Anaerobic dehalogenation	unsupported
Suicide plasmids	120,000
Metabolic pathway recruitment	103,000
Extramural support (2,4,5-T degradation)	50,000
Hazardous Waste Engineering Laboratory Cincinnati, Ohio	
2,4,5-T degradation	
White rot fungus	
P. Chrysosporium	
Yeast	
PCB degradation	
Plants	
Biofilm reactor	
Leachate slurries	
Guidance document	
Robert S. Kerr Environmental Research Laboratory Ada, Oklahoma	1,413,300
TCE-degradation	
Models for spilled hydrocarbons	
Models for soil cleanup	
Stanford demonstration project	
Water Engineering Research Laboratory Cincinnati, Ohio	
Genetics of methanogens	
Microbial binding proteins	
Prediction of biodegradation	
Environmental Research Laboratory Athens, Georgia	
Anaerobic activity	14,000
Screening of indigenous organisms	70,000
Office of Exploratory Research (Extramural Grants)	482,571[b]
SITE Program	
Evaluation of PCB degradation	0[c]
TOTAL	3,496,871

[a]Excludes projects whose primary purpose is risk assessment.
[b]Figure represents one half of the total of five two-year grants, $965,143.
[c]Anticipated expenditures for FY88 are approximately $200,000.

SOURCE: Office of Technology Assessment, 1988.

and subsurface materials to identify processes that may be used in biological treatment systems;

- develop new biosystems for the treatment of pollutants, including genetically engineered and naturally selected microorganisms, consortia, and bioproducts;
- determine, evaluate, and demonstrate the engineering factors necessary for the application of biological agents to detoxify or destroy pollutants;
- determine the environmental fate of and effects of and the risks involved in the use or release of biological agents or their products developed to detoxify or destroy pollutants;
- develop means to mitigate adverse consequences resulting from the accidental or deliberate release of biotechnology products developed for pollution control; and
- transfer information on the technology to promote its use (101).

EPA is marginally supporting various programs with long-term potential benefits. Program managers believe that many programs will have long-term payoff, but cannot be completed without additional funding. These programs include:

- genetically engineered anaerobic dehalogenators of chlorinated aromatics, which could be ready for commercialization and field application at Superfund sites within 3 to 4 years;
- immobilized ligninases isolated from white rot fungi, which could be used to oxidize chlorinated hydrocarbons within 3 to 5 years; and
- plant root fungi, which could be used to concentrate toxic metals from contaminated soils by 1992 (100).

EPA Research Laboratories. The Environmental Research Laboratory in Gulf Breeze, FL, is one of EPA's leading laboratories for research involving new biotechnologies. While their effort is focused on risk assessment for the release of novel organisms, several projects are focused on developing biotechnology to cleanup hazardous waste. Gulf Breeze has projects to develop organisms to degrade trichloroethylene (TCE), to investigate the biology of complex waste sites; to investigate the anaerobic dehalogenation of hydrocarbons; to develop a '(suicide plasmid" that would cause the organism to die once degradation of a target compound was complete; and to facilitate the transfer of metabolic pathways into new organisms. In addition, Gulf Breeze is supporting research at the University of Illinois on the microbial degradation of 2,4,5-T, an active ingredient in Agent Orange (66). The total cost of these projects was $373,000 in fiscal year 1987 (66).

The Hazardous Waste Engineering Research Laboratory (HWERL) in Cincinnati, OH, is the principal laboratory of the Office of Research and Development responsible for developing and evaluating technologies for hazardous waste control. HWERL has been supporting projects in biodegradation for several years (28). HWERL, along with Gulf Breeze, supports the 2)4,5-T work at the University of Illinois (66). HWERL also has a small biosystems program investigating the enzymes of the white rot fungus, which have been shown to reduce dioxins and other pollutants (39) and supports a range of extramural research projects in biodegradation. In addition, it is the lead laboratory in the Biosystems Initiative, described previously. The cost of these projects was about $850,000 in fiscal year 1987 (28).

The Robert S. Kerr Laboratory in Ada, OK, focuses on groundwater research and has identified a microbial process that may be capable of cleaning up TCE from aquifers and groundwater. The process is different from that used for TCE-degradation at the Gulf Breeze labs. This process relies on the ability of a group of naturally occurring microbes, called methanotrophs, to cooxidize trichloroethylene and a variety of other halogenated organic compounds when methane, propane, or natural gas is added. Researchers at Kerr Laboratory have demonstrated degradation using soil columns and are conducting field and laboratory tests in cooperation with Stanford University, the University of Oklahoma, and the Air Force (57). In the field tests, the bacteria degraded nearly 30 percent of the TCE in groundwater (l). Kerr Laboratory is also working to develop mathematical models for biosystems cleanups. The laboratory also has an active anaerobic biodegradation effort underway, as well as studies on subsurface microbiology.

EPA's Water Engineering Research Laboratory in Cincinnati supports several bioremediation projects. Projects include the study of the genetics of methanogens, with the long-term objective to improve the rate and reliability of anaerobic digestion; the study of microbial binding proteins, particularly the metallothionein enzyme, which is known to bind cadmium; and a study on the prediction of the biodegradation of toxic compounds based on structure-activity relationships. The laboratory has also worked to develop a protocol for evaluating bioaugmentation projects (106).

Microbial research at the Environmental Research Laboratory at Athens, GA, is concerned primarily with the fate of environmental pollutants. Research on the degradation of chlorinated compounds in anaerobic environments is an integral component of the in-house program. In addition, a 3-year cooperative program with New York University is looking at the stability of anaerobic consortia capable of degrading various organic pollutants (81).

EPA's Office of Exploratory Research supported five extramural research projects (all at universities) related to biological remediation systems for a 2-year total of $965)143 in 1986 and 1987. The extramural projects all involve in situ treatment, thus the emphasis on biological systems, and include bioenrichment with hydrogen peroxide, biodegradation of chlorinated aliphatic solvents, and other projects (17).

From 1982 to 1987, EPA also selected biodegradation for 4 Superfund sites, out of a total of 41 sites for which treatment technologies were used (102),

Cleanup of Federal Waste Sites

The Federal Government has been a substantial waste generator. The civilian Federal agencies have at least 1,882 potentially hazardous waste sites but have studied only half of them to determine whether cleanup is necessary. Of these, 1,326 sites belong to the Department of Energy (DOE); 1,061 of these were for the production of nuclear materials and weapons (5).

The Department of Defense (DoD) has also been a substantial waste generator, reporting 400 to 800 sites, which need remediation at a cost of $5 to $10 billion over the next 10 years. DoD supports a substantial research and development effort related to hazardous waste, in part as a response to the requirement to clean up its hazardous waste sites via their Installation Restoration Program (IRP), which is analogous to the Superfund. The DoD is collaborating with the EPA and DOE to develop demonstration projects at a total cost of $5 million (DoD's share is $1.953 million). A few of these relate to the use of biotechnology, according to the DoD program manager (27).

A significant portion of the DoD waste resulted from airplane engine cleaning solvents, airplane fuel spills, paint stripping, and nuclear waste. The Environics Division of the Research and Development Directorate of the Air Force is the service's principal laboratory for environmental research and development and maintains the lead in the DoD for biotechnology research and coordination with other Federal programs. The laboratory's work focuses on hazardous waste reduction; recovery and treatment of polluted soils; polluted groundwater treatment; and alternative energy sources. The laboratory funds both intramural projects (some funded jointly by EPA) and extramural programs, both in laboratories and at IRP sites. One project is funded jointly with DOE as a Small Business Innovation Research award.

The laboratory's research includes a wide-ranging program of 24 projects encompassing air and groundwater, containment chemistry, microbial degradation, and waste treatment. In-house research and program management staff includes 38 people, 9 holding doctorates, with an annual budget of $8 million. Six of the 24 projects use biological treatment of contaminants and several other projects provide background information to support biological methodology. Laboratories at Tyndall Air Force Base are investigating biological degradation of TCE, dioxin, and organometallies. They are attempting to isolate and modify micro-organisms capable of degrading contaminants, with particular interest in mixed culture systems and enhancement of conditions. Recombinant DNA modifications are used in the laboratory. Among other innovative technology projects is a contract to Cornell University to examine the use of ***aphrons,*** small (about 25 microns), stable bubbles, which serve both as a transport mechanism and as an oxygen source for biological agents to clean up aquifers.

Other Federal Research Activities

The Department of Energy (DOE) supports some research and development related to biotechnological approaches to waste management. Within the Deep Subsurface Microbiology Research Program, DOE supports projects involving 15 intramural and extramural researchers investigating microbial community structure and the factors that control microbial habitats and reactions at depths of 30 to thousands of meters, the depth of many of the nation's largest aquifers. These projects are aimed at in situ degradation ***of*** organic contaminants at DOE sites. The Ecological Research Division supports research on microbial fermentation of cellulose to methane and carbon dioxide, the mechanisms by which plants metabolize metals, and other plant processes. Through the DOE Small Business Innovation Research Program, five projects support bioenvironmental research and development. No studies on cost analyses of conventional versus biotechnological approaches could be identified at DOE. DOE supports some interagency research efforts with the Air Force and maintains contact with the Los Alamos and Idaho National Engineering Laboratories (31)95,96).

DOE's Idaho National Engineering Laboratories maintains a biotechnology unit with an interdisciplinary program, comprising molecular genetics, bioseparations, bioprocessing, biohydrometallurgy, and biochemical engineering focused on basic and applied microbiology relating to recovery of metal from ore and waste streams, removal of sulfur and metals from fossil fuels, solubilization and gasification of fossil fuels, degradation of toxic organic materials, and production and separation of proteins and carbohydrates. The laboratory is supported by the Department of Interior as well as by DOE (41,97).

The National Aeronautics and Space Administration (NASA), through its Controlled Ecological Life Support System (CELSS), supports work on waste conversion as part of its focus on maintaining life processes and recycling technology in closed systems. NASA also supports a limited amount of research on the metabolism of exotic organisms that live in unusual habitats, such as sea vents (6).

The National Science Foundation (NSF) supports about 10 projects for $2.5 million that are directly related to bioremediation of waste. One is a research center at the University of California at Los Angeles focused on engineering for hazardous substance control. The foundation also sponsors workshops on bioremediation (68).

The Department of Interior conducts several in-house projects on the bioleaching of manganese and supports research at Morehouse College on metallo-resistance. Interior, through the Bureau of Mines, also provides $500,000 to the Idaho National Engineering Laboratory for projects related to metals extraction and recovery and bio-assisted minerals processing.

The National Institute of Environmental Health Sciences has funded four research projects under authority granted in the Superfund Amendments and Reauthorization Act of 1986. Funds come from the Superfund Trust Fund. Research includes developing organisms tailored to degrade toxic waste, developing combinations of appropriate organisms, designing reactors, and defining operating conditions.

The Department of Education, through its Division of Higher Education Incentives, provided a grant of $488,000 in fiscal year 1987 to the University of Tennessee at Knoxville to establish a Center for Environmental Biotechnology, whose research focuses on environmental hazardous waste degradation (78).

Private Sector Investment

Investment by the private sector in waste management technologies is driven by regulation. Without regulation there would be little incentive for the major waste generators to minimize or clean up waste, and there would be a much smaller market for waste management services and technologies. Regulations also determine which waste cleanup technologies can be used.

New and stricter regulations are driving up the cost of traditional waste management services. Service providers who can find cheaper or safer methods of disposing wastes will have a clear advantage in today's markets. However, most waste management companies are small and cannot afford substantial R&D expenditures. Some of the large waste generators, on the other hand, can and do support R&D efforts. Biotechnology appears to be the subject of strong interest from venture capitalists interested in investing in waste management, although precise figures are not available.

An entire industry of small and not-so-small companies has sprung up to respond to the regulatory environment. These environmental companies range from engineering-oriented to biotechnically oriented, and a few are mixed. Twenty-one companies are listed in a recent directory as providing biological treatment services for hazardous waste material management. While substantially more companies are listed for chemical treatment and incineration (49 and 64 respectively) (40), the number of companies in biological treatment is significant and probably growing. A total of 65 companies, including waste generators, have been identified as involved in waste management biotechnology (see app. D).

The level of R&D investment in biotechnology for waste management by each company varies significantly. OTA has obtained figures for 1986 R&D expenditures from 10 of these 65 companies, which range from zero at one company to about $3 million at another. The 10 companies are all service providers, not waste generators.

In certain subspecialties, a substantial portion of even the most basic aspects of biotechnology research is supported by private funds. For example, the most advanced work on genes to degrade several of the chemicals listed on the National Priority List sites is being conducted in industrial laboratories.

Research and development costs at waste management companies cannot be separated cleanly from engineering costs and other costs of doing business. The industry's role is to provide scientific and engineering services, much of which could be considered R&D. Since each waste site is unique, each requires some original research before the best cleanup technology can be identified. In many cases, new engineering solutions must be developed that will enable degradation to occur while controlling the release or migration of contaminants. Some companies enter into research and development limited partnerships, in which the waste management company and the client (typically a waste generator) share ownership of whatever techniques are developed. R&D is thus based on what the client will buy.

RESEARCH AND DEVELOPMENT NEEDS

Research needs include microbial ecology, physiology, genetic expression and control, site engineering site characterization, and feasibility studies. Numerous microbes with degradative capabilities have been identified and isolated. Other metabolic capabilities are still needed, however, and the isolation of **new strains of micro-organisms is an important area of research.** Many of those organisms already identified need refining and enhancement to be useful for field application; these activities require research investment. Site engineering and feasibility studies require that the metabolizes produced be understood and that the migration of compounds in the environment be predictable. Knowledge of surfactants and emulsifiers, often needed for the microbes to react with the target compound, is also required.

Basic Research Needs

The lack of knowledge of microbial physiology and ecology is a major scientific stumbling block from the standpoints of efficacy, efficiency, economics, and environmental safety. While molecular biology has had several decades of stable funding, microbial ecology has lagged behind, and suddenly the need for information about microbial ecosystems is acute (24,71).

Identifying and selecting organisms to alter waste continues to be an important area of endeavor. While many of microorganisms have been located with propitious characteristics, other organisms with even more advantages can probably be found (22,63).

Interest in anaerobic degradation has increased in recent years. Anaerobes may circumvent the need for oxygen in some applications. Anaerobes are also likely to produce novel reactions, such as the dechlorination reaction described previously, that could provide key steps in degrading target chemicals. Knowledge of the biology and genetic manipulation of anaerobes, however, lags behind that for aerobes (61),

For wastes that resist degradation by known organisms, engineering new organisms might be appropriate. To genetically engineer microbes for specific waste problems, the genes of interest and their control elements need to be identified. As can be seen in table 11-1, many degradative organisms have been identified, but the pathways, enzymes, and genes involved in degradation are often unknown. Genes for other processes, such as production of energy, surfactants, and emulsifiers also need to be identified if these traits are to be genetically engineered.

Applied Research Needs

Keeping the degrading organism alive and active and providing access to target compounds are the two most basic needs in applying biotechnology to pollution problems. Strategies include providing oxygen and nutrients and exploiting microbial symbiosis. The potential advantages of creating engineered organisms with all the required capabilities versus creating mixed communities of specialist organisms are not known. However, researchers are reluctant to deal with engineered organisms when alternatives are available.

Biodegradation requires making the target compound available to the organisms. Pollutants frequently occur attached to solids, mixed as hydrophobic and hydrophilic waste, or as low molecular weight semi-volatiles in groundwater, often making them inaccessible to microbes. Molecular and engineering strategies to make pollutants available to organisms are poorly developed. Surfactants and emulsifiers produced by naturally occurring organisms should be investigated.

Information on the characteristics and measurement of pollutants is only partly developed (76, 104), especially with regard to chemically similar compounds, called congeners. Extreme and varied concentrations of compounds, known as hot spots, complicate assessment (76). Measuring groundwater contamination in situ, for example, is problematic (70).

Careful and thorough demonstration and evaluation studies of bioremediation techniques are also required. Current practice frequently relies on a single line of evidence for the disappearance of one pollutant and relies on samples from a few spots in an uneven mixture. Lack of information in this area leads to a lack of credibility regarding the effectiveness of cleanup efforts. In at least one case of putative degradation, for example, an organism was thought to have degradative capabilities because a target compound disappeared from the medium in which the organism was grown. It turned out the compound had not been degraded but absorbed by the organism, a useful property but not one that actually degrades or detoxifies the pollutant. Comparative data regarding the relative efficacy economics, and environmental safety of biotechnical **versus conventional methods are seriously lacking.**

BARRIERS TO DEVELOPMENT OF THE TECHNOLOGY

Although the potential advantages of innovations in biotechnological approaches for waste management are recognized, it is generally accepted that the development of biotechnology products for hazardous waste management is lagging behind product development in other sectors of the biotechnology industry, such as pharmaceutical and agricultural applications (104). The barriers to innovative applications lie in several areas, including funding, regulations, personnel, and economics.

Funding and Programmatic Implementation

Technical and scientific barriers to biotechnological waste management are discussed in the previous section on “Research and Development Needs.” Knowledge gaps result from uneven funding for basic research in certain fields, such as microbial ecology, and from the fragmented and uncoordinated nature of funding for R&D of biotechnological approaches to hazardous waste management. Funding is fragmented for these reasons:

- Neither the public nor the private sector takes responsibility for funding basic or generic applied research in important areas. Microbial physiology and ecology have not been well supported by any agency or other resource.
- Technical and scientific advancement of the waste field is strongly linked to regulations and to the funding programs of a single lead

agency. The amount of funding available supports only a few researchers and projects, and the available funding lacks a coherent program to develop the necessary technical and scientific base in identified critical areas. In addition, EPA's enforcement efforts and regulatory authority were in a state of flux in the early 1980s (23), which reduced the private sector's incentives for research (26).

problems With Private Investment

Waste management companies face a range of disincentives to investing in bioengineered approaches to waste management, from regulatory uncertainty to R&D expense. Waste management companies focus development efforts on technologies that have been developed by others and that can be purchased ready for commercialization, thus avoiding the R&D risk.

Waste companies also favor technologies in which the treatment times and effluent concentrations can be predicted with reliability, even when the costs are much higher, in order to reduce potential liability. Bioengineered approaches are almost never as predictable, at least with current knowledge, as nonbiological alternatives (8,9).

Since much of the waste management and cleanup market involves the remediation of complex waste sites, companies may prefer to invest in techniques better suited to handle complex mixtures of waste. Complex waste sites are currently beyond the capabilities of biotechnological waste management techniques.

Problems With Public Investment

Both researchers and industrial managers say they believe that EPA does not provide clear management with regard to developing the field scientifically and does not manage its programs from a broad enough perspective to give appropriate weight to biotechnological approaches. Questions about the best implementation of the program have been raised many times (59)63,92)104)) but have not been resolved. These include:

- developing clear standards,
- developing assessment technologies to support implementing these standards, and
- conducting clear, comparative studies of biotechnology and conventional approaches to hazardous waste treatment to answer the efficacy, economic, and safety questions that face the field,

Regulatory programs have strongly directed the patterns of the research conducted. As EPA's experience has been largely with land disposal and incineration, some believe that staff at the agency are poorly equipped to deal with biotechnology. For example, access to federally designated sites for demonstration projects appears weighted in favor of nonbiological approaches (26,79,86,102). While many innovative nonbiological methods are also worthy of testing and evaluation, biological methods clearly will not be fully developed without additional testing and evaluation.

Funding by the EPA is insufficient and comparatively unstable. The Agency is funding high-quality in-house research, but at a level too low to develop biotechnology's potential for hazardous waste detoxification. Unstable funding of extramural projects, in particular, prevents initiating long-term projects, For example, a leading researcher with demonstrated ability to produce organisms tailored to degrade toxic substances believes that he could develop a microorganism that would degrade dioxin (21)64). Development would require stable funding of $125)000 per year for 5 to 7 years. The project would require a high-quality and experienced postdoctoral fellow, but the researcher cannot in good faith recruit such a person because even funded projects are subject to cancellation by EPA. This project is not suitable for students or postdoctoral fellows for a short period, both because it requires dedicated expertise and because researchers at those stages in their careers must have projects that produce results in time for them to write a dissertation or secure a job. In addition, the toxicity of the compound being examined suggests the need for designated research facilities with researchers specially trained in necessary safety measures. In short, this project and others like it will not be accomplished without a stable, long-term commitment (21).

Until the critical research areas are addressed and performance standards are clearly established, cleanup claims offered by

individual companies will lack credibility. Only studies conducted without conflict of interest will resolve efficacy, economic, and environmental safety questions.

Regulation

Regulation dictates what must be cleaned up, how clean it must be, and which cleanup methods may be used. Thus, regulation determines what is developed for the waste field. Currently, regulation favors the use of contained cleanup methods and the use of naturally occurring, indigenous organisms. Although no recombinant organism is ready for field trial, such an organism eventually will be ready. However, fears of regulatory barriers are discouraging researchers from investigating genetic engineering as a means of developing novel, potentially beneficial, organisms.

Individual companies have reported great difficulty in getting approval or support from EPA for biological approaches or even access to sites for demonstration projects. Company research directors and presidents have complained that EPA is biased toward nonbiological approaches, and that it is extremely difficult to get regulators to consider biological techniques (26,86).

In addition, HSWA defines land disposal to include land treatment. Thus, with the onset of the Land Disposal Restrictions, RCRA-listed hazardous waste cannot be placed in or on the land for eventual biodegradation, even as a landfarming experiment, unless a petitioner can prove the waste will not migrate for as long as it remains hazardous (109). Furthermore, obtaining the RCRA permit takes approximately 4 years. A research, development, and demonstration permit can be obtained in about 8 months (109).

Regulatory resistance to bioremediation stems from a variety of factors. One prominent problem is that bioremediation techniques have been oversold in the past, so the field lacks credibility. Many applications are new and do not have any history of effectiveness. A second problem involves time limitations: certain biological applications take longer than incineration or excavation. Although they may be cheaper or more thorough, bioremediation techniques may be passed over due to the desire to address the problem as quickly as possible. In addition, performance standards have been established only for land disposal and incineration. Finally, engineers, who are often the regulators, may not be familiar with the biology involved in these cleanup systems. EPA frequently does not have adequate data for evaluating biotechnology. No regulations require companies to compile and submit data on alternative technologies. Also, companies often do not want anyone to know they have a hazardous waste problem and thus do not make their information public.

Personnel

A serious impediment to greater use of bioremediation techniques is the lack of technical understanding by regulatory enforcement personnel (86) and by many EPA contractors involved in waste management. In some cases, officials have relied on bioremediation data from the 1970s (86) despite the numerous advances made in recent years. Small businesses with waste problems have testified to the reluctance on the part of regulators to accept the possibility that biodegradation works (86).

Despite testimony from EPA scientists that biodegradation may be applicable to many Superfund sites (86), EPA apparently does not see the need to increase its expertise in biology in the Superfund program. Personnel with expertise in the biological sciences constituted 25 of 1,643 full-time equivalents in the Superfund program in fiscal year 1986, or 2 percent of the total (105). An EPA work force planning study concluded that Superfund's current and strong orientation toward engineering and physical/environmental sciences was appropriate for future field operations. On the basis of anticipated trends and changes in the program, the following occupation areas were reported to be underrepresented among Superfund staff: hydrology, geology, and procurement and contracts (105). Such a conclusion, if heeded, does not indicate that EPA will increase its expertise in the biological sciences and bioremediation techniques. An EPA administrator, however, has suggested that if the agency develops the necessary knowledge base in biotechnology, it is possible that EPA would begin to recommend microbial degradation for RCRA corrective actions (109).

Since the application of biotechnology to hazardous waste management is relatively new, the infrastructure for training is underdeveloped, The field requires bioengineers with interdisciplinary training in chemical and civil engineering and biology as well as hydrogeologists with expertise in biology (16). Additional personnel with expertise in microbial ecology are also required (see ch. 8) (87). Underfunding of microbial ecology in the past has led to a shortage of expertise.

Economic Uncertainty

Whether conventional methods or biotechnologies offer more economic potential to clean up waste is under debate. Small, young biotechnology firms, in particular, cannot afford the high risk, uncertain-payoff R&D efforts required to develop new technologies. Uncertainty about regulations and liability also discourages some firms from pursuing innovative technologies. EPA is, however, addressing this problem with a small conference to bring entrepreneurs, venture capitalists, and EPA regulators together to clarify potential opportunities (43).

Theoreticians argue that, once the technology is developed, biological degradation is always cheaper than chemical treatment or burning because the organism supplies a catalyst that works at ambient temperatures and, if designed well, generates its own energy, without additives. Others argue that the high up-front cost of biotechnology research and development brings the economic projections for the two approaches closer together.

The need to develop specific solutions for each in situ cleanup adds to economic uncertainty. Each site has unique characteristics, ranging from the type of contaminant to soil porosity to local politics. A treatment system developed for one site may or may not be applicable to other sites.

ISSUES AND OPTIONS FOR CONGRESSIONAL ACTION

ISSUE 1: Should research and development in biotechnology for waste management be stimulated?

Option 1.1: Take no action.

Biotechnology for waste management has suffered in recent years from various funding and institutional barriers. Its development is in a relative state of infancy compared with that of biotechnology in pharmaceuticals and agriculture. However, public and private interest in biotechnology for waste management is increasing without specific congressional action. Nonetheless, without some initiatives, key research barriers are likely to go unaddressed for several years or longer and adequate efficacy and efficiency demonstrations will not be carried out. Without specific action, the relevant agencies, in particular EPA, are not likely to develop in-house scientific and managerial expertise for the assessment and regulation of bioremediation techniques.

Option 1.2: Increase funding for research in biodegradation activities.

Increased funding for research and development in biotechnology for waste management could bring attention to key research areas that are currently bottlenecks in the application of the technology, such as microbial physiology and ecology, genetic engineering of anaerobes, and the development of specific degradative pathways for key persistent compounds, such as dioxin. Additional funds could also facilitate demonstration and evaluation projects, which require support from government or other disinterested parties if results are to be unbiased and credible. For example, with funds to increase its expertise in biology, EPA would be better able to evaluate potential research and development projects, demonstration projects, and cleanup projects that use innovative biological methods. Without such funding, EPA will continue to fund only certain high priority projects, and at relatively low levels.

Option 1.3: Provide funds for training programs in disciplines related to waste management biotechnology, including microbial ecology, biohydrogeology, and environmental engineering with emphasis in biotechnology

The successful development of waste management biotechnology requires a wide range of expertise in the waste management industry, in State and Federal regulatory agencies, and in research universities. The predominance of personnel with experience in land disposal and incineration in the waste management field has left biological and other innovative technologies with an uphill battle for acceptance. A training strategy should be two-pronged, including both training current engineers in biology and the training of new environmental engineers in bioremediation.

Option 1.4: Clarify and enforce existing regulations regarding hazardous waste cleanup and disposal.

The claim has been made that existing regulations are not being fully or uniformly enforced. Standards for cleanup are also not always clear and can change. Enforcing existing regulations will ensure that more cleanup technologies are used and will create incentives for developing more cost-effective technologies.

Option 1.5: Establish more stringent standards to require permanently remediated and ecologically sound sites.

Regulations drive the field of waste management. The current Superfund program established cleanup standards that emphasize permanent remedies. However, RCRA standards are generally less stringent. Also, performance standards for bioremediation are less well developed than those for incineration or land disposal. Such performance standards need to be clarified. Regulations requiring permanent disposal of wastes could spur the development of technologies that will detoxify or destroy wastes and leave products that can be returned to use in the environment.

ISSUE 2: Is the management and regulation of biological cleanup technologies adequate and appropriate?

Option 2.1: Take no action.

In the current system, both basic and applied R&D is supported by a variety of public and private organizations, including several Federal agencies and private companies, However, neither the public nor private sector takes responsibility for many basic and strategically important research and development areas. As a result, there is no coherent program for overall management of R&D and no strategy for developing the field. Key research barriers are not being addressed, and demonstration and evaluation projects are lacking. A limited number of innovative technologies are being attempted through programs such as the Superfund Innovative Technology Evaluation (SITE) program. without some management initiative, the field cannot be expected to develop in a timely manner. EPA's Biosystems Initiative is a first step in this direction, but EPA's principal focus is regulation, not research and development. The present system may, however, protect the public from excessive or irresponsible applications of bioengineered cleanup approaches that could exacerbate, without solving, the problem.

Option 2.2: Establish an interagency coordinating body to create strategies for developing biological cleanup technologies.

Currently, EPA, the National Science Foundation, the National Institutes of Health, the Department of Interior, the Department of Energy, and the Department of Defense have significant programs related to bioengineered waste cleanup technologies. An interagency coordinating group could identify major gaps in the research and work to prevent unnecessary duplication of efforts by Federal agencies. This option would not necessarily cost the government more money, nor would it ensure more money would go for research.

Option 2.3: Clarify regulations on the environmental application of genetically engineered organisms.

The private sector favors activities involving nonengineered organisms due to the uncertainty surrounding regulations for engineered organisms. While nonengineered organisms are frequently effective and appropriate, opportunities may be missed if genetic engineering is not explored. Congress could encourage the use of genetically engineered organisms for waste cleanup by resolving the issues of deliberate release of novel organisms. Adopting this option could lead to the creation of organisms with important new degradative capabilities.

SUMMARY AND CONCLUSIONS

Biotechnology offers real possibilities for providing permanent solutions to hazardous and nonhazardous environmental wastes. Most of its potential, however, remains unrealized due to technical, institutional, economic, and perceptual barriers. Progress is being made in each of these areas.

Interest in waste management biotechnology is growing in the public and private sector, but the field continues to suffer from a lack of personnel in regulatory agencies and in the waste management industry who understand biology. The field suffers from a credibility problem brought about partly by earlier claims that were not supported by scientific fact and partly by the inertia in the waste management community that favors traditional methods of land disposal and incineration. In addition, much fundamental research is needed if biological techniques are to achieve high rates of destruction on a broad range of toxic wastes. There is a strong need for demonstrating and evaluating innovative bioremediation techniques. EPA has begun to move in this direction with the SITE program and the proposed Biosystems Initiative.

Many chemical waste sites are amenable to biodegradation, and practical applications are underway. Many other potential applications require substantial amounts of research and development before field trials can be attempted. Much work with naturally occurring or laboratory-selected strains can proceed, avoiding the perceptual and regulatory problems of using genetically engineered micro-organisms.

However, current applications of biological remediation techniques are generally suited to a limited range of pollutants in accessible conditions. Expanding the range of wastes amenable to bioremediation and degrading those wastes in the environments in which they occur to the very low concentrations needed may, ultimately, require genetic engineering. Engineering such microbes will require a substantial investment in R&D, and may face significant problems of public perception. Regardless of how the organisms are derived, thorough knowledge of waste ecology, of degradative intermediates and end products, and of the migration of both the organisms and the chemicals is needed.

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