Bioremediation for Marine Oil Spills

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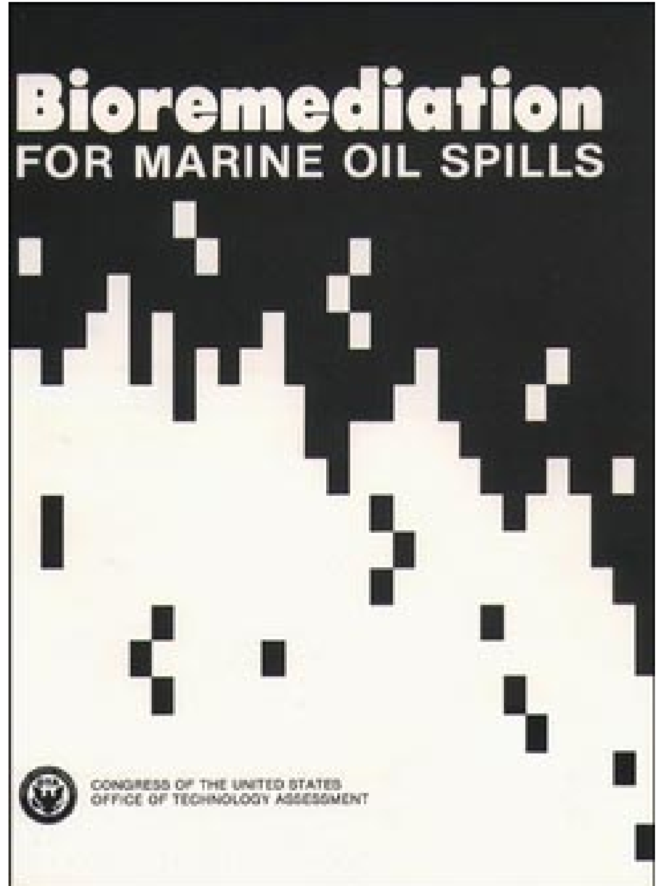

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Foreword

If anything positive has resulted from the massive *Exxon Valdez oil* spill, it is that this environmental calamity increased the Nation's awareness of the shortcomings of its capability to fight oil spills and prompted it to take steps to correct this situation. In the 2 years since this spill occurred, Congress passed major oil pollution legislation (the Oil Pollution Act of 1990); private industry established and provided significant funding for the Marine Spill Response Corp.; and Federal agencies reevaluated their responsibilities, revised contingency plans, and took steps to improve response technologies.

In addition to concern with refining the efficiency and reliability of the existing response technologies, both the public and private sectors have sought to develop innovative new technologies for responding to spills. bioremediation is one such technology. Although the possibility of using the capabilities of oil-degrading microorganisms to accelerate the natural biodegradation of oil has been discussed for years, it is only recently that some of the practical problems associated with this idea have begun to be addressed. The *Exxon Valdez* spill, in particular, gave researchers a rare opportunity to evaluate the feasibility of using bioremediation as an oil spill countermeasure.

This OTA background paper evaluates the current state of knowledge and assesses the potential of bioremediation for responding to marine oil spills. Our basic message is a dual one: we caution that there are still many uncertainties about the use of bioremediation as a practical oil spill response technology; nevertheless, it could be appropriate in certain circumstances, and further research and development of bioremediation technologies could lead to enhancing the Nation's capability to fight marine oil spills. The request for this study came from Senator Ted Stevens, a member of OTA's Technology Assessment Board, and the senior senator from the State of Alaska, where bioremediation was tested on beaches polluted by oil from the *Exxon Valdez.*

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INTRODUCTION

In March 1990 *the* Office of Technology Assessment (OTA) published *Coping With an Oiled Sea: An Analysis of Oil Spill Response Technologies. The* study was prompted in part by the alarm and concern that followed the Nation's largest oil spill to date, the n-million-gallon spill in Prince William Sound, Alaska, caused by the grounding of the *Exxon Valdez.* OTA concluded that if the damage *from* such pollution incidents is to be minimized in the future, major improvements are required in the organization and the technologies employed to fight oil spills. OTA evaluated the state-of-the-art and the potential for improvement of the most widespread technologies-mechanical containment and cleanup methods-and assessed what appeared to be the most promising alternatives to mechanical methods-in particular, the use of chemical dispersants and in situ burning.

One "new" technology about which OTA had little to say in its initial study was bioremediation—the use of microorganisms to accelerate the degradation of oil or other environmental contaminants (box A). Degradation of oil by microorganisms is one of the most important long-term *natural processes* for removal of oil from the marine environment.[1] Given enough time-at least several years, for example, for oil stranded on beaches-some microorganisms are capable of at least partially cleaning environments polluted with oil. Because bioremediation is a potentially significant method for mitigating the damage caused by marine oil spills,[2] techniques to accelerate and improve the efficiency of this natural process have had a number of proponents over the past two decades. Although considerable research has been conducted in the last 10 years on the development of bioremediation techniques, imporant questions remain about their effectiveness, possible unintended side effects of their use, and their importance in comparison with more conventional oil spill response technologies. Research concerning these issues has been given new momentum, in large part because the *Exxon Valdez* accident stimulated a general search for more effective methods to fight oil spills. Consequently, the data that those directly responsible for fighting oil spills--e. g., on-scene coordinators-will need before they are willing or able to include biological techniques in their arsenal of countermeasures are beginning to be developed.

This study examines the potential of bioremediation technologies to clean up marine oil spills and to minimize the damage they cause. Thus, the study evaluates a small, but highly visible, subset of the many possible applications of bioremediation technologies to environmental problems. Among the other applications for which bioremediation is being considered or is currently in use are: 1) treatment of nontoxic liquid and solid wastes, 2) treatment of toxic or hazardous wastes, 3) treatment of contaminated groundwater, and 4) grease decomposition.[3] Although recent marine oil spills and bioremediation efforts have called attention to the potential of bioremediation as an oil spill response technology, some of these other applications, in particular the treatment of hazardous waste, appear to have greater potential. Officials at approximately 135 hazardous waste sites, for example, are now either considering, planning, or **operating** full-scale bioremediation systems.[4]

Consideration of the applicability of bioremediation to oil spills is not new. Investigation of microbial degradation of oil dates to at least 1942, when the American Petroleum Institute began to subsidize research on the topic.[5] Considerable basic knowledge about factors that affect *natural* biodegradation, about the kinds of hydrocarbons capable of being degraded, and about the species and distribu-

[1] National Research Council, *Oil in the Sea: Inputs, Fates, and Effects* (Washington DC: National Academy Press, 1985), P. 290.

[2] The term "marine oil spills" is here defined to include spills at sea, in bays and estuaries, on beaches, and in environments such as salt marshes in contact with the sea.

[3] Several OTA studies have addressed various aspects of these topics. See, for example, *Commercial Biotechnology: An International* Analysis, OTA-BA-218 (Washington DC: U.S. Government Printing Office, 1984); *New Developments in Biotechnology, OTA-BA-360 (Washington* DC: U.S. Government Printing Office, July 1988); *Coming Clean: Superfund Problems Can Be Solved,* OTA-ITE-433 (Washington DC: U.S. Government Printing Office, October 1989); *and Biotechnology in a Global Economy* (Washington, DC: U.S. Government Printing Office, expected publication date mid-1991).

[4] U.S. Environmental Protection Agency, bioremediation Action Committee, Summary of Nov. 7, 1990 meeting.

[5] C.E. Zobell, "Microbial Degradation of Oil: Present Status, Problems, and Perspectives," in *The Microbial Degradation of Oil Pollutants (proceedings* of a workshop at Georgia State University, Atlanta, December 1972), D.G. Ahearn and S.P. Meyers (eds.), 1973.

Box A—bioremediation v. Biodegradation

Biodegradation refers to the natural process whereby bacteria or other microorganisms alter and break down organic molecules into other substances, such as fatty acids and carbon dioxide.

bioremediation is the act of adding materials to contaminated environments, such as oil spill sites, to cause an acceleration of the natural biodegradation process.

Fertilization is the bioremediation method of adding nutrients, such as nitrogen and phosphorus, to a contaminated environment to stimulate the growth of indigenous microorganisms. This approach is also termed nutrient enrichment.

Seeding refers to the addition of microorganisms to a spill site. Such microorganisms mayor may not be accompanied by nutrients. Current seeding efforts use naturally occurring microorganisms. Seeding with genetically engineered microorganisms (GEMs) may also be possible, but this approach is not now being considered for remediating oil spills.

tion of the microorganisms involved in biodegradation had already been developed by the early 1970s. For example, the Office of Naval Research sponsored about 15 basic and applied research projects in the late 1960s and early 1970s on oil biodegradation as part of the charge to the Navy at that time to take the lead in mitigating marine oil pollution. In 1972, a workshop on the microbial degradation of oil pollutants was sponsored by the Office of Naval Research, the U.S. Coast Guard, and the U.S. Environmental Protection Agency (EPA).[6] The 1980s were a period of rapid advances in knowledge of the genetics and molecular biology of bacterial degradation of different hydrocarbons, and of renewed interest in the microbial ecology of pollution-stressed environments.

Much progress has been made in applying basic knowledge of biodegradation to cleaning up terrestrial and enclosed sites polluted with oil. As long ago as 1967, contractors employed bioremediation to improve the quality of 800,000 gallons of oily wastewater remaining in the bilge tanks of the *Queen Mary* after it was permanently moored in Long Beach Harbor.[7] City officials approved the discharge of this bilge water 6 weeks after treatment. More importantly, a large number of refineries, tank farms, and transfer stations now employ in situ bioremediation to restore land contaminated by accidental spills of fuel oil or other hydrocarbons.[8]

Much less progress has been made with respect to the practical problems of applying bioremediation technologies to marine oil spills, although advocates have suggested their use in the wake of several major spills. The problems associated with using bioremediation technologies in marine environments are fundamentally different from those associated with land-based applications. Although bioremediation of oil-contaminated soil is one of the fastest growing uses of this technology, bioremediation applications on land have all been accomplished in closed or semi-enclosed environments where microorganisms have little or no competition and where conditions can be closely controlled and monitored. The marine environment is a dynamic, open system that is much less susceptible to control, and many additional variables exist to compound the difficulties of applying bioremediation techniques. One of the most important series of tests and the first large-scale application of a bioremediation technology in a marine setting was conducted between 1989 and 1991 by the EPA, Exxon, and the State of Alaska on the Prince William Sound beaches fouled by oil from the *Exxon Valdez.*

SUMMARY AND FINDINGS

Biodegradation is a natural process, and there is no question that, with enough time, microorganisms can eliminate many components of oil from the environment. The central concern of this study is whether bioremediation technologies can accelerate this natural process enough to be considered practical, and, if so, whether they might find a niche as replacements for, or adjuncts to, other oil spill response technologies. The key findings from this OTA study are summarized below:

- **The usefulness of bioremediation for marine oil spills is still being evaluated, and their**

[6] D.G. Ahearn and S.P. Meyers (eds.), *The MicrobialDegradation of Oil Pollutants* (proceedings of a workshop at Georgia State University, Atlanta, December 1972) (Baton Rouge, LA: Center for Wetland Resources, Louisiana State University, 1973).

[7] Applied Biotreatment Association, "Case History Compendium," November 1989, p. 34. The compendium also Contains other examples of the use of bioremediation technologies to address environmental problems.

[8] T.G. Zitrides, "Bioremediat.ion Comes of Age," *Pollution Engineering,* vol. XXII, No. *5, May* 1990, pp. 59-60.

Photo credit: Environmental Protection Agency

Prince William Sound, Alaska, site of the extensive bioremediation experiments carried out by the Environmental Protection Agency, Exxon, and the State of Alaska.

ultimate importance relative to other oil spill response technologies remains uncertain. Recent research and field testing of bioremediation technologies on oiled beaches has produced some encouraging, if not altogether conclusive, results. Nevertheless, technologies other than bioremediation (especially mechanical ones) are likely to remain the mainstay of the Nation's response arsenal for now. In certain non-emergency situations (e.g., for cleaning lightly to moderately oiled beaches), bioremediation could be employed as a primary technology. Mechanical methods, dispersants, and possibly in situ burning will most likely remain more appropriate technologies for the immediate response to spills at sea.

- Potential bioremediation approaches for marine oil spills fall into three major categories: 1) stimulation of indigenous microorganisms through addition of nutrients (fertilization), 2) introduction of special assemblages of naturally occurring oil-degrading microorganisms (seeding), and 3) introduction of genetically engineered microorganisms (GEMs) with special oil-degrading properties. Stimulation of indigenous organisms by the addition of **nutrients is the approach that has been tested most rigorously. This approach is viewed by many researchers as the most promising one for responding to most types of marine spills.** Recent experiments suggest that rates of biodegradation in most marine environments are constrained by lack of nutrients rather than by the absence of oil-degrading microbes. The introduction of microbes *might be* beneficial in areas where native organisms grow slowly or are unable to degrade a particular hydrocarbon. However, the effectiveness of this approach has not yet been demonstrated. The wide availability of naturally occurring microorganisms capable of degrading components of petroleum will likely deter consideration of GEMs for remediating marine oil spills. Moreover, greater research and development needs, regulatory hurdles, and public perception problems will remain obstacles to the near-term use of GEMS even if they could prove useful for degrading some recalcitrant components of petroleum.

- bioremediation technologies for beach cleanup have thus far received the most attention. Experiments conducted by EPA, Exxon, and the State of Alaska on cobble beaches fouled by oil from the *Exxon Valdez* indicated that the *addition of nutrients* at least doubled the natural rate of biodegradation. The efficacy of commercial *microbial products in* remediating beaches is not yet known. Limited EPA field tests using two microbial products on heavily weathered oil in Alaska were inconclusive. Additional field experiments are required on other types of beaches that involve different oils and different climatic and marine conditions.

- bioremediation **has not yet been demonstrated to be an effective response to "at-sea" oil spills. The** necessary studies have not been done, in part because of the difficulty of conducting controlled experiments and monitoring in the open ocean. Limited applications have been made in the Gulf of Mexico, but they have not provided definitive data on effectiveness. The design and validation of open ocean protocols to test products are necessary before the efficacy of bioremediation at sea can be determined or widely accepted. Even if bioremediation proves effective in some situations, other, quicker-acting alternatives may be preferable as primary response tools.

- bioremediation may have a role in settings such as salt marshes and sensitive ecosystems where the use of mechanical or other approaches might do more harm than good. Just as for open water spills, however, appropriate protocols need to be developed for testing and applying bioremediation technologies in these situations, and more research is required to prove their effectiveness.
- No significant adverse impacts related to the use of bioremediation technologies for oil spill cleanup have been identified in recent field applications. Effects that have been measured have been short-lived and minor. On beaches, in particular, bioremediation may be a less intrusive approach than other alternatives. However, experience with bioremediation in marine settings is limited, and it is premature to conclude that the use of bioremediation technologies will be safe in all circumstances.
- Regulatory controls to ensure the safe use of bioremediation appear adequate, and there appear to be no significant Federal regulatory obstacles to the greater use of bioremediation technologies, except those using GEMs. However, **more development and testing of both fertilization and seeding technologies are needed before on-scene coordinators or others responsible for oil spill cleanup would be comfortable advocating their use.** Most decisionmakers prefer more traditional methods, and usually are not willing to experiment during a real spill. bioremediation technologies for response to marine oil spills, although promising, are still in the experimental phase. One regulatory change that could help stimulate development of both bioremediation and other oil spill response technologies is for the Federal Government to allow occasional controlled oil spills for research and development purposes.
- If additional research confirms the effectiveness of bioremediation and leads to the development of more reliable technologies, oil spill decisionmakers will have to be educated about the efficacy of various techniques, the advantages and disadvantages of their use, and the availability of materials and expert assistance. Preliminary efforts to accomplish this have recently been undertaken by EPA. However, before detailed bioremediation contingency plans can be developed, uncertainties about the effectiveness of bioremediation must be addressed. Detailed plans, when and if necessary, would require such information as the oil-degrading capabilities of microorganisms indigenous to a particular area, the characteristics of the oil most likely to be spilled in that area, environmental factors constraining oil biodegradation, and the circumstances under which the use of bioremediation technologies would be appropriate or allowed.
- EPA, through its bioremediation Action Committee and research labs, and with the assistance of the National Environmental Technology Applications Corp., is developing protocols to determine the efficacy and toxicity of bioremediationproducts in a variety of settings. Testing products against such protocols would provide decisionmakers with the kind of data needed to determine whether these products could be used in response to marine oil spills.
- A research program to expand the present knowledge of biodegradation mechanisms, and to improve bioremediation technologies and the means of applying them to marine oil spills, appears to be justi.tied. Redirecting an appreciable fraction of available marine oil spill research funds to bioremediation does not, however, seem warranted. Efforts to improve other oil spill prevention and response technologies are also important, and funding is limited. Improving methods for enhancing the growth and activity of petroleum-degrading bacteria is important, as is the development of better analytical techniques for measuring and monitoring effectiveness, and field validation of laboratory work. Government and industry should be encouraged to coordinate their research efforts and to share as much information as possible.

BACKGROUND

Evaluating the effectiveness of bioremediation technologies is complicated by several factors. First, biodegradation is only one of the processes at work removing petroleum from the marine environment; to understand the effect of this process on oil removal, one must know the effects of other processes. Second, petroleum is not the simple material many people presume it to be; rather, it contains thousands of compounds. Some of these are easily biodegraded; others are relatively resistant to biodegradation. Third, a large number-not just one or a few-of species of microorganisms are responsible for biodegradation, and these species have evolved many metabolic pathways to degrade oil. Although the general mechanisms of biodegradation are known, many details remain to be filled in.

Underlying these complications are the basic issues of what constitutes clean and how long one is willing to wait for results. These are both political and scientific issues. Can a beach be considered clean, for example, if its surface appears clean but close examination reveals oil below the surface, or if unsightly but relatively less harmful constituents of oil, such as hard-to-degrade asphalt, remain on the beach after bioremediation is used? How much of an improvement over natural biodegradation rates must bioremediation technologies offer before their use would be warranted? A rate increase of a factor of 2 or more would be significant for a spill that might otherwise persist for 5 or more years, but much less so for a spill that might be naturally degraded in less than a year.

The Fate of Oil in the Marine Environment

The fate of petroleum in marine ecosystems has been intensively studied.[9] Crude oil and petroleum distillate products introduced to the marine environment are immediately subject to a variety of physical and chemical, as well as biological, changes (figure 1).[10] Abiological weathering processes include evaporation, dissolution, dispersion, photochemical oxidation, water-in-oil emulsification, adsorption onto suspended particulate material, sinking, and sedimentation. Biological processes include ingestion by organisms as well as microbial degradation.[11] These processes occur simultaneously and cause important changes in the chemical composition and physical properties of the original pollutant, which in turn may affect the rate or effectiveness of biodegradation.

The most important weathering process during the first 48 hours of a spill is usually evaporation, the process by which low- to medium-weight crude oil components with low boiling points volatilize into the atmosphere. Evaporation can be responsible for the loss of one- to two-thirds of an oil spill's mass during this period,[12] with the loss rate decreasing rapidly over time.[13] Roughly one-third of the oil spilled from the *Amoco Cadiz*, for example, evaporated within the frost 3 days. Evaporative loss is controlled by the composition of the oil, its surface area and physical properties, wind velocity, air and sea temperatures, sea state, and the intensity of solar radiation.[14] The material left behind is richer in metals (mainly nickel and vanadium), waxes, and asphaltenes than the original oil.[15] With evaporation, the specific gravity and viscosity of the original oil also increase. For instance, after several days, spilled crude oil may begin to resemble Bunker C (heavy) oil in composition.[16]

None of the other abiological weathering processes accounts for as significant a proportion of the losses from a spill. For example, the dissolving, or dissolution, of oil in the water column is a much less important process than evaporation from the perspective of mass lost from a spill; dissolution of even a few percent of a spill's mass is unlikely. Dissolution is important, however, because some water-soluble fractions of crude oil (e.g., the light aromatic compounds) are acutely toxic to various marine organisms (including microorganisms that may be able to degrade other fractions of oil), and their impact on the marine environment is greater than mass balance considerations might imply.[17]

9For example, see references listed in National Research Council, *Oil in the Sea: Inputs, Fates, and Effects* (Washington, DC: National Academy Press, 1985), pp. 335-368; and in G.D. Floodgate, "The Fate of Petroleum in Marine Environments,' R.M. Atlas (cd.), *Petroleum Microbiology (New York, NY: Macmillan* Publishing Co., 1984), pp. 355-397.

10National Research Council, op. cit., footnote 1, p. 270.

11J.R. Payne and G.D. McNabb, Jr., "Weathering of Petroleum in the Marine Environment,' *Marine Technology Society Journal,* vol. 18, No. 3, 1984, p. 24.

12National Research Council, op. cit., footnote 1, p. 276.

13Floodgate, op. cit., footnote 9, p. 362.

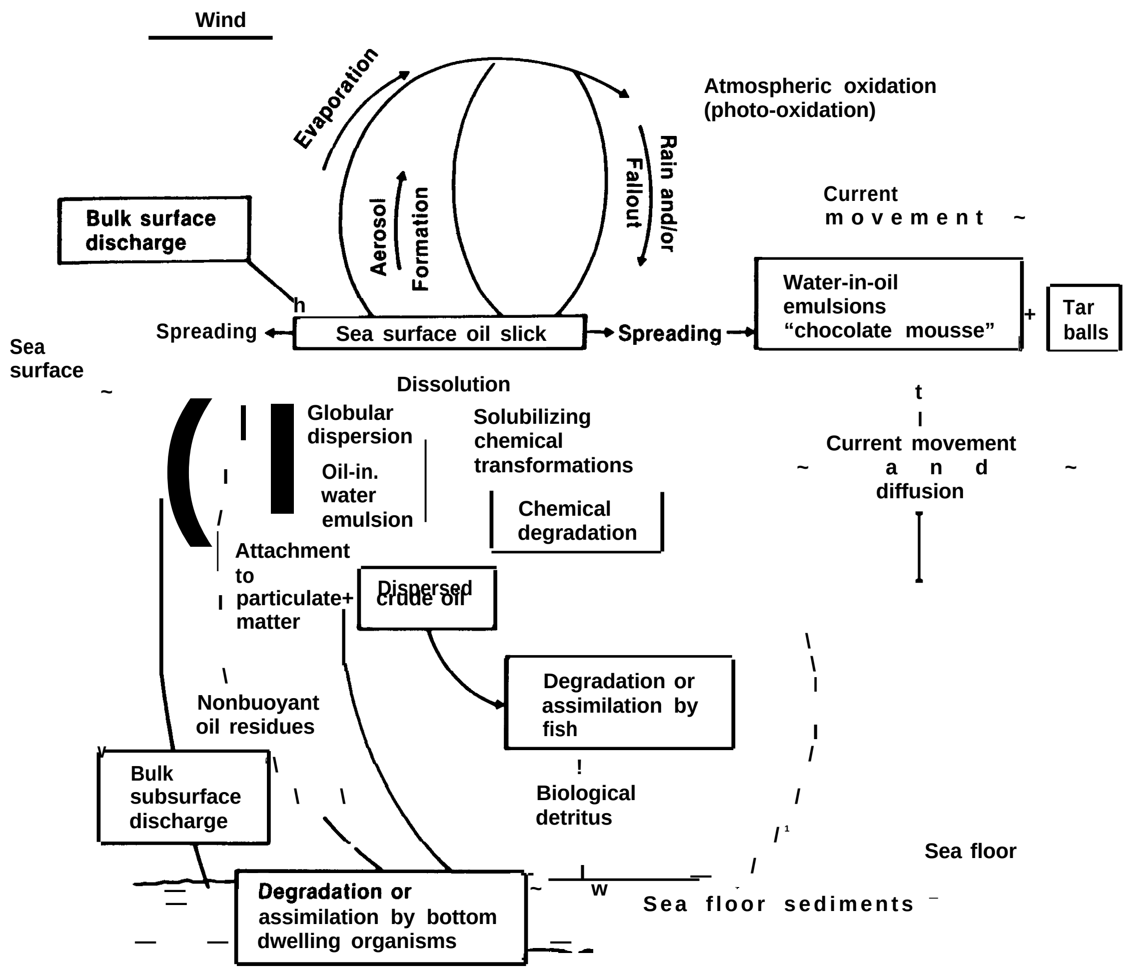
14Payne and McNabb, op. cit., footnote 11, p. 26.

15Floodgate, op. cit., footnote 9, p. 362.

16J.E. Mielke, "Oil in the Ocean: The Short- and Long-Term Impacts of a Spill," Congressional Research Service, 90-356 SPR, July 24, 1990, p. 11.

17National Research Council, op. cit., footnote 1, pp. 277-278.

Figure I—Schematic of Physical, Chemical, and Biological Processes



SOURCE: National Research Council, *Oil in* the Sea: Inputs, Fates, and Effects (Washington, DC: National Academy Press, 1985), p. 271. Adapted from R. Burwood and G.C. Speers, "Photo-oxidation as a Factor in the Environmental Dispersal of Crude Oil," Estuarine Coastal *Marine Science, vol. 2, 1974,* pp. 117-135.

Dispersion, the breakup of oil and its transport as small particles from the surface to the water column, is an extremely important process in the disappearance of a surface slick.[18] Dispersion is controlled largely by sea surface turbulence: the more turbulence, the more dispersion. Chemical dispersants have been formulated to enhance this process. Such dispersants are intended as a first-line defense against oil spills that threaten beaches and sensitive habitats such as salt marshes and mangrove swamps. Although used widely in other countries, dispersants have had trouble being accepted in the United States. The National Research Council has generally approved their use,[19] but effectiveness and, to a lesser degree, toxicity remain concerns. Dispersed oil particles are more susceptible to biological attack than undispersed ones because they have a greater exposed surface area. Hence, dispersants may enhance the rate of natural biodegradation.[20]

Water-in-oil emulsions, often termed "mousse," are formed when seawater, through heavy wave

[18] Payne and McNabb, op. cit., footnote 11, p. 30.

[19] National Research Council, Marine Board, *Using Oil Dispersants on the Sea* (Washington, DC: National Academy Press, 1989).

[20] R.R. Colwell and J.D. Walker, "Ecological Aspects of Microbial Degradation of Petroleum in the Marine Environment" *CRC Critical Reviews in Microbiology,* September 1977, p. 430.

action, becomes entrained with the insoluble components of oil. Such emulsions can form quickly in turbulent conditions and may contain 30 to 80 percent water.[21] Heavier or weathered crudes with high viscosities form the most stable mousses. Mousse will eventually disperse in the water column and/or be biodegraded, but may first sink or become stranded on beaches. A water-in-oil emulsion is more difficult for microorganisms to degrade than oil alone.[22] Mousse formation, for example, has been suggested as a major limiting factor in petroleum biodegradation of the Ixtoc I and *Metula* spills, probably because of the low surface area of the mousse and the low flux of oxygen and mineral nutrients to the oil-degrading microorganisms within it.[23]

Natural biodegradation is ultimately one of the most important means by which oil is removed from the marine environment, especially the nonvolatile components of crude or refined petroleum (see below). In general, it is the process whereby microorganisms (especially bacteria, but yeasts, fungi, and some other organisms as well) chemically transform compounds such as petroleum hydrocarbons into simpler products. Although some products can actually be more complex, ideally hydrocarbons would be converted to carbon dioxide (i.e., mineralized), nontoxic water-soluble products, and new microbial biomass. The mere disappearance of oil (e.g., through emulsification by living cells) technically is not biodegradation if the oil has not actually been chemically transformed by microbes.[25] The ideal may be difficult to reach, particularly in a reasonably short time, given the recalcitrance of some petroleum fractions to biodegradation (discussed below) and the many variables that affect its rate and extent. Man-made bioremediation technologies are intended to improve the effectiveness of natural biodegradation.

Biodegradation and the Chemical Nature of Petroleum

Far from being a homogeneous substance, crude oil is a complex mixture of thousands of different chemical compounds. In addition, the composition of each accumulation of oil is unique, varying in different producing regions and even in different unconnected zones of the same formation.[26] The composition of oil also varies with the amount of refining. Significantly, the many compounds in oil differ markedly in volatility, volubility, and susceptibility to biodegradation. Some compounds are readily degraded; others stubbornly resist degradation; still others are virtually nonbiodegradable. The biodegradation of different petroleum compounds occurs simultaneously but at very different rates. This leads to the sequential disappearance of individual components of petroleum over time and, because different species of microbes preferentially attack different compounds, to successional changes in the degrading microbial community .27 Since components of petroleum degrade at different rates, it is difficult and misleading to speak in terms of an overall biodegradation rate.

Petroleum hydrocarbons can, in general, be divided into four broad categories: saturates, aromatics, asphaltenes, and resins.[28] Saturated hydrocarbons-those with only single carbon-carbon bonds—usually constitute the largest group. Of these, the normal or straight-chain alkane series is the most abundant and the most quickly degraded. Compounds with chains of up to 44 carbon atoms can be metabolized by microorganisms, but those having 10 to 24 carbon atoms (C_{10}-C_{24}) are usually the easiest to metabolize. Shorter chains (up to about C_{12}) also evaporate relatively easily. Only a few species can use C_1-C_4 alkanes; C_5-C_9 alkanes are degradable by some microorganisms but toxic to

21 Mielke, Op. cit., footnote 16, P. 12.

22 K. Lee and E.M. Levy, ''Biodegradation of Petroleum in the Marine Environment and Its Enhancement,'' in *Aquatic Toxicology and Water Quality Management,* J.O. Nrigau and J.S.S. Lakshminarayana (eds.) (New York, NY: John Wiley & Sons, 1989), p. 221.

23 R.M. Atlas, ''Biodegradation of Hydrocarbons in the Environment,'' in *Environmental Biotechnology,* G.S. Omenn (cd.), (New York, NY: Plenum Press, 1988), p. 214.

24 National Research Council, Op. Cit., footnote 1, P. 290.

25 J.J. Cooney, "Microbial Ecology and Hydrocarbon Degradation" paper presented at the Alaska Story Symposium, Cincinnati, OH, Sept. 17-18, 1990., p. 2.

26 National Research Council, op. cit., footnote 1, p. 17.

27 Atlas, op. cit., footnote 23, p. 212.

28 J.G. Leahy and R.R. Colwell, "Microbial Degradation of Hydrocarbons in the Environment" *Microbiological Reviews,* September 1990, p. 305.

others.[29] Branched alkanes are usually more resistant to biodegradation than normal alkanes but less resistant than cycloalkanes (naphthenes)-those alkanes having carbon atoms in ringlike central structures.[30] Branched alkanes are increasingly resistant to microbial attack as the number of branches increases. At low concentrations, cycloalkanes may be degraded at moderate rates, but some highly condensed cycloalkanes can persist for long periods after a spill.[31] Light oils contain 10 to 40 percent normal alkanes, but weathered and heavier oils may have only a fraction of a percent. Heavier alkanes constitute 5 to 20 percent of light oils and up to 60 percent of heavier oils.[32]

Aromatic hydrocarbons are those characterized by the presence of at least one benzene (or substituted benzene) ring. The low-molecular-weight aromatic hydrocarbons are subject to evaporation and, although toxic to much marine life, are also relatively easily degraded. Light oils typically contain between 2 and 20 percent light aromatic compounds, whereas heavy oils contain 2 percent or less.[33] As molecular weight and complexity increase, aromatics are less readily degraded. Thus, the degradation rate of polyaromatics is slower than that of monoaromatics. Aromatics with five or more rings are not easily attacked and may persist in the environment for long periods.[34] High-molecular-weight aromatics comprise 2 to 10 percent of light oils and up to 35 percent of heavy oils.[35]

The asphaltic fraction contains compounds that either are not biodegradable or are degraded very slowly.[36] One of the reasons that tar, which is high in asphaltenes, makes an excellent road paving material is because it is slow to degrade. Tar balls, like mousse, are difficult to degrade because their low surface area restricts the availability of oxygen and other nutrients. Resins include petroleum compounds containing nitrogen, sulfur, and/or oxygen as constituents. If not highly condensed, they may be subject to limited microbial degradation. Asphaltenes and resins are difficult to analyze and, to date, little information is available on the biodegradability of most compounds in these groups.[37] Light oils may contain about 1 to 5 percent of both asphaltenes and resins; heavy or weathered oils may have up to 25 percent asphaltenes and 20 percent resins.[38]

To summarized biodegradation *rates are* typically highest for the saturates, followed by the light aromatics, with high-molecular-weight aromatics, asphaltenes, and resins exhibiting extremely low rates of degradation.[39] As a spill weathers, its composition changes: the light aromatics and alkanes dissolve or evaporate rapidly and are metabolized by microorganisms. The heavier components that are harder to degrade remain. Weathered Prudhoe Bay oil contains about 10 percent low-molecular-weight alkanes, 45 percent high-molecular-weight alkanes, 5 percent light aromatics, 20 percent high-molecular-weight aromatics, 10 percent asphaltenes, and 10 percent resins.[40]

Departures from the typical pattern of biodegradation, however, have been noted by some researchers. For example, extensive losses of asphaltenes and resins have been observed in some cases. The microbial degradation of these relatively recalcitrant fractions has been ascribed to co-oxidation.[41] In this process, a normally refractory hydrocarbon may be partially degraded in the presence of a second readily degraded hydrocarbon. Clearly, degradation rates depend on many factors, and generalizations are difficult to make. One conclusion, however, seems reasonable: no crude oil is subject to complete biodegradation, and claims that all of a light oil or

29 Atlas, op. cit., footnote 23, p. 212.

30 Cooney, op. cit., footnote 25.

31 Atlas, op. cit., footnote 23, p. 212.

32 M. Fingas, Environment Canada, personal communication, Oct. 5, 1990.

33 Ibid.

34 National Research Council, op. cit., footnote 1, p. 293.

35 Fingas, op. cit., footnote 32.

36 Cooney, op. cit., footnote 25, p. 3.

37 Cooney, op. cit., footnote 25, p. 3; and National Research Council, op. cit., footnote 1, p. 295.

38 Fingas, op. cit., footnote 32.

39 Leahy and Colwell, op. cit., footnote 28, p. 305.

40 Fingas, op. cit., footnote 32.

41 Leahy and Colwell, op. cit., footnote 28, p. 306.

more than 50 percent of a heavy oil can be biodegraded in days or weeks are highly suspect.[42]

Microbial Processes and the Degradation of Petroleum

Despite the difficulty of degrading certain fractions, some hydrocarbons are among the most easily biodegradable naturally occurring compounds. Altogether, more than 70 microbial genera are known to contain organisms that can degrade petroleum components (table 1). Many more as-yet-unidentified strains are likely to occur in nature.[43] Moreover, these genera are distributed worldwide. All marine and freshwater ecosystems contain some oil-degrading bacteria. No one species of microorganism, however, is capable of degrading all the components of a given oil. Hence, many different species are usually required for significant overall degradation.[44] Both the quantity and the diversity of microbes are greater in chronically polluted areas. In waters that have not been polluted by hydrocarbons, hydrocarbon-degrading bacteria typically make up less than 1 percent of the bacterial population, whereas in most chronically polluted systems (harbors, for example) they constitute 10 percent or more of the total population.[45]

Microorganisms have evolved their capability to degrade hydrocarbon compounds over millions of years. These compounds are a rich source of the carbon and energy that microbes require for growth. Before that carbon is available to microorganisms, however, large hydrocarbon molecules must be metabolized or broken down into simpler molecules suitable for use as precursors of cell constituents. The activity of microorganisms at a spill site is governed by the organisms' ability to produce enzymes to catalyze metabolic reactions. This ability is, in turn, governed by their genetic composition. Enzymes produced by microorganisms in the presence of carbon sources are responsible for attacking the hydrocarbon molecules. Other enzymes are utilized to break down hydrocarbons further.[%] Lack of an appropriate enzyme either prevents attack or is a barrier to complete hydrocarbon degradation.

Table I—Major Genera of Oil-Degrading Bacteria and Fungi

Bacteria	*Fungi*
Achrornobacter	Allescheria
Acinetobacter	Aspergillus
Actinomyces	Aureobasidium
Aeromonas	Botrytis
Alcaligenes	Candida
Arthrobacter	Cephalosporium
Bacillus	Cladosporium
Beneckea	Cunninghamella
Brevebacterium	Debaromyces
Coryneforms	Fusarium
Erwinia	Gonytrichum
Flavobacterium	Hansenula
Klebsiella	Helminthosporium
Lactobaoillus	Mucor
Leumthrix	Oidiodendrum
Moraxella	Paecylomyces
Nocardia	Phialophora
Peptococcus	Penicillium
Pseudomonas	Rhodosporidium
Sarcina	Rhodotorula
Spherotilus	Saccharomyces
Spirillum	Saccharomycopisis
Streptomyces	Scopulariopsis
Vibrio	Sporobolomyces
Xanthomyces	Torulopsis
	Trichoderma
	Trichosporon

SOURCE: G.D. Floodgate, "The Fate of Petroleum in Marine Ecosystems," *Petroleum Microbiology,* R.M. Atlas (ad.) (New York, NY: Macmillan Publishing Co., 1984), p. 373.

The complex series of steps by which biodegradation occurs constitutes a metabolic pathway. Many different enzymes and metabolic pathways, not all of which can be found in any single species, are required to degrade a significant portion of the hydrocarbons contained in petroleum. (Thus, advocates of using specially selected mixtures of microorganisms to bioremediate oil spills or of creating, through recombinant DNA technology, genetically engineered organisms are motivated in part by the desire to combine all the requisite enzymes and pathways.[47])

Knowledge of the numerous metabolic pathways involved in the breakdown of hydrocarbons is far from complete. Additional research characterizing the microbiology and population dynamics of bac-

[42] Cooney, op. cit., footnote 25, P- 3.

[43] Floodgate, op. cit., footnote 9, p. 372.

[44] Lee and Levy, op. cit., footnote 22, pp. 217-243.

[45] Cooney, op. cit., footnote 25, p. 1.

[46] Applied Biotreatment Association, "The Role of Biotreatment of Oil Spills," rev. 2, August 1990, p. 4.

[47] Atlas, op. cit., footnote 23, P. 213.

terial species capable of degrading oil is critical to understanding the biodegradation process.

Environmental Influences on Biodegradation

Environmental variables can also greatly influence the rate and extent of biodegradation. Variables such as oxygen and nutrient availability can often be manipulated at spill sites to enhance natural biodegradation (i.e., using bioremediation). Other variables, such as salinity, are not usually controllable. The great extent to which a given environment can influence biodegradation accounts for some of the difficulty in accurately predicting the success of bioremediation efforts. Lack of sufficient knowledge about the effect of various environmental factors on the rate and extent of biodegradation is another source of uncertainty.

Oxygen

Oxygen is one of the most important requirements for microbial degradation of hydrocarbons. However, its availability is rarely a rate-limiting factor in the biodegradation of marine oil spills. Microorganisms employ oxygen-incorporating enzymes to initiate attack on hydrocarbons. Anaerobic degradation of certain hydrocarbons (i.e., degradation in the absence of oxygen) also occurs, but usually at negligible rates. Such degradation follows different chemical paths, and its ecological significance is generally considered minor.[48] For example, studies of sediments impacted by the *Amoco Cadiz* spill found that, at best, anaerobic biodegradation is several orders of magnitude slower than aerobic biodegradation.[49] Oxygen is generally necessary for the initial breakdown of hydrocarbons, and subsequent reactions may also require direct incorporation of oxygen. Requirements can be substantial; 3 to 4 parts of dissolved oxygen are necessary to completely oxidize 1 part of hydrocarbon into carbon dioxide and water.[50]

Oxygen is usually not a factor limiting the rate of biodegradation on or near the surface of the ocean, where it is plentiful and where oil can spread out to provide a large, exposed surface area. Oxygen is also generally plentiful on and just below the surface of beaches where wave and tide action constantly assist aeration. When oxygen is less available, however, the rates of biodegradation decrease. Thus, oil that has sunk to the sea floor and been covered by sediment takes much longer to degrade. Oxygen availability there is determined by depth in the sediment, height of the water column, and turbulence (some oxygen may also become available as the burrowing of bottom-dwelling organisms helps aeration).[51] Low-energy beaches and fine-grained sediments may also be depleted in oxygen; thus, the rate of biodegradation may be limited in these areas. Pools of oil are a problem because oxygen is less available below their surfaces. Thus, it may be preferable to remove large pools of oil on beaches, as was done in Alaska, before attempting bioremediation.

Nutrients

Nutrients such as nitrogen, phosphorus, and iron play a much more critical role than oxygen in limiting the rate of biodegradation in marine waters. Several studies have shown that an inadequate supply of these nutrients may result in a slow rate of biodegradation.[52] Although petroleum is rich in the carbon required by microorganisms, it is deficient in the mineral nutrients necessary to support microbial growth.[53] Marine and other ecosystems are often deficient in these substances because non-oil-degrading microorganisms (including phytoplankton) consume them in competition with the oil-degrading species. Also, phosphorus precipitates as calcium phosphate at the pH of seawater. Lack of nitrogen and phosphorus is most likely to limit biodegradation, but lack of iron or other trace minerals may sometimes be important. Iron, for instance, is more limited in clear offshore waters than in sediment-rich coastal waters.[54]

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[48] Leahy and Colwell, op. cit., footnote 28, p. 307.

[49] D.M. Ward, R.M. Atlas, P.D. Boehm, and J.A. Calder, "Microbial Biodegradation and the Chemical Evolution of *Amoco Cadiz* Oil Pollutants," *Ambio*, vol. IX, No. 6, 1980, pp. 279.

[50] Lee and Levy, op. cit., footnote 22, p. 224.

[51] Cooney, op. cit., footnote 25, p. 4.

[52] Lee and Levy, op. cit., footnote 22, p. 223.

[53] Atlas, op. cit., footnote 23, p. 216.

[54] Ibid.

Phofo credit: Exxon Corp.

Workman applying fertilizer to the cobble beaches of Prince William Sound.

Scientists have attempted to adjust nutrient levels (e.g., by adding nitrogen- and phosphorus-rich fertilizers) to stimulate biodegradation of petroleum hydrocarbons. This is the experimental bioremediation approach used recently on about 110 miles of beaches in Prince William Sound, Alaska. Researchers have also experimented with alternative methods of *applying* nutrients. Given the necessity of keeping nutrients in contact with oil, the method of application is itself likely to be an important factor in the success of bioremediation.

Temperature

The temperature of most seawater is between –2 and 35°C.[55] Biodegradation has been observed in this entire temperature range, and thus in water temperatures as different as those of Prince William Sound and the Persian Gulf. The rates of biodegradation are fastest at the higher end of this range and usually decrease—sometimes dramatically in very cold climates-with decreasing temperature. One experiment showed that a temperature drop from 25 to 5°C caused a tenfold decrease in response.[56] At low temperature, the rate of hydrocarbon metabolism by microorganisms decreases.[57] Also, lighter fractions of petroleum become less volatile, thereby leaving the petroleum constituents that are toxic to microbes in the water for a longer time and depressing microbial activity. Petroleum also becomes more viscous at low temperature. Hence, less spreading occurs and less surface area is available for colonization by microorganisms. In temperate regions, seasonal changes in water temperature affect the rate of biodegradation, but the process continues year-round.[58]

Other Factors

Several variables, including pressure, salinity, and pH may also have important effects on biodegradation rates. Increasing pressure has been correlated with decreasing rates of biodegradation; therefore, pressure may be very important in the deep ocean. Oil reaching great ocean depths degrades very slowly and, although probably of little concern, is likely to persist for a long time.[59]

Microorganisms are typically well adapted to cope with the range of salinities common in the world's oceans. Estuaries may present a special case because salinity values, as well as oxygen and nutrient levels, are quite different from those in coastal or ocean areas. However, there is little evidence to suggest that microorganisms are adversely affected by other than hypersaline environments.[60]

Extremes in pH affect a microbe's ability to degrade hydrocarbons. However, like salinity, pH does not fluctuate much in the oceans-it remains between 7.6 and 8. l—and does not appear to have an important effect on biodegradation rates in most marine environments. In salt marshes, however, the pH maybe as low as 5.0, and thus may slow the rate of biodegradation in these habitats.[61]

55Floodgate, op. cit., footnote 9, p. 381.

56Ibid.

57Leahy and Colwell, op. cit., footnote 28, p. 307.

58Floodgate, op. cit., footnote 9, p. 381-

59J.R. Schwarz, J.D. Walker, and R.R. Colwell, "Deep-sea Bacteria: Growth and Utilization of Hydrocarbons at Ambient and In Situ Pressure," *Applied Microbiology, vol. 28, 1974,* pp. 982-986.

60Lee and Levy, op. cit., footnote 22, p. 225.

61Leahy and Colwell, op. cit., footnote 28, p. 308.

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Table 2—bioremediation: Potential Advantages and Disadvantages

Advantages:
- Usually involves only minimal physical disruption of a site
- No significant adverse effects when used correctly
- Maybe helpful in removing some of the toxic components of oil
- Offers a simpler and more thorough solution than mechanical technologies
- Possibly less costly than other approaches

Disadvantages:
- Of undetermined effectiveness for many types of spills
- May not be appropriate at sea
- Takes time to work
- Approach must be specifically tailored for each polluted site
- Optimization requires substantial information about spill site and oil characteristics

SOURCE: Office of Technology Assessment, 1991.

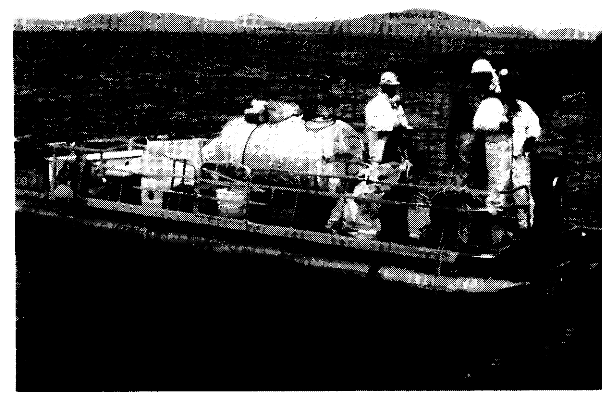


Photo credit: Exxon Corp.

One of the shallow draft boats used by Exxon to apply oleophilic fertilizer to Prince William Sound beaches.

General Advantages and Disadvantages of bioremediation

bioremediation technologies have several attributes that, depending on the situation and type of site may support their use in responding to some oil spills (table 2).[62] First, bioremediation usually involves minimal physical disruption of a site. This attribute is especially important on beaches where other available cleanup technologies (e.g., high- and low-pressure spraying, steam cleaning, manual scrubbing, and raking of congealed oil) may cause additional damage to beach-dwelling biota.[63] Application of oleophilic (i.e., oil seeking) fertilizers during the 1989-90 Alaska bioremediation experiments was accomplished largely from shallow draft boats located just off the beach. Second, bioremediation technologies appear to have no or only minor and short-lived adverse effects when used correctly. Although research on possible negative impacts is continuing, there is so far little evidence to suggest that potential problems would be significant.

Third, bioremediation may be useful in helping remove some of the toxic components of petroleum (e.g., low-molecular-weight aromatic hydrocarbons) from a spill site more quickly than they might otherwise be removed by evaporation alone.[64] Fourth, bioremediation of oil spills is accomplished on-site, and offers a simpler and more thorough solution to polluted areas. In contrast, hot water spraying of an oiled beach, for example, flushes some surface oil back into the water, and this oil must then be recovered by skimmers. The recovered oil-and-water mixture must be separated, and the oil disposed of or recycled. Also, a significant amount of mechanical equipment and logistical capability is required to deal with a large spill.

Because bioremediation equipment and logistics are usually simpler and less labor intensive, costs *may be* lower than for other techniques. At the same time, the total cost of cleanup is the more important concern, and where bioremediation is used as an adjunct or secondary technology, total costs—as well as total benefits--could be greater. The costs of monitoring bioremediation must also be considered.

bioremediation technologies have several general disadvantages. Although bioremediation may work faster-potentially much faster—than natural biodegradation, it cannot produce significant ***short-term*** results. If beaches are threatened by a large offshore spill, for instance, bioremediation is probably not appropriate as an initial defensive measure. In this circumstance, it would usually be more appropriate to get the oil out of the water as quickly as possible or, failing this, to disperse or burn it before it drifts onto beaches. bioremediation takes

[62] These attributes are discussed in the Applied Biotreatment Association, "BriefmgPaperonthe Role ofbioremediation of Oil Spills," rev. 2, August 1990.

[63] M.S. Foster, J.A. Tarpley, and S.L. Dearn, "To Clean or Not lb Clean: The Rationale, Methods, and Consequences of Removing Oil From Temperate Shores," *The Northwest Environmental Journal*, vol. 6, 1990, pp. 105-120.

[64] J. Glaser, Environmental Protection Agency, Risk Reduction Engineering Laboratory, personal communication, Feb. 20,1991.

too much time to work as a primary response measure for such a threat.

Second, the bioremediation approach must be specifically tailored to each polluted site. Bioremediation technologies are not, and are unlikely soon to become, off-the-shelf technologies that can be used with equal effectiveness in every locale. Although other oil spill response technologies are subject to this same constraint, the advance knowledge needed for bioremediation technologies is greater. Advance knowledge of, for example, the efficiency of the bacteria indigenous to an area in degrading oil, the availability of rate-limiting nutrients, and the susceptibility of the particular spilled crude oil or refined product to microbial attack is required, so prespill planning will be important.

Finally, the public is still unfamiliar with bioremediation technologies. Although public attitudes toward "natural" solutions to environmental problems are generally favorable, the lack of knowledge about microorganisms and their natural role in the environment could affect the acceptability of their use.[65] Before bioremediation technologies are likely to be widely used, their efficacy and safety will have to be convincingly demonstrated and communicated to the public.

ALTERNATIVE bioremediation TECHNOLOGIES

bioremediation technologies for responding to marine oil spills may be divided into three discrete categories: 1) nutrient enrichment, 2) seeding with naturally occurring microorganisms, and 3) seeding with genetically engineered microorganisms (GEMs) (table 3).

Table 3-Principal Features of Alternative bioremediation Approaches

Nutrient enrichment:
- Intended to overcome the chief limitation on the rate of the natural biodegradation of oil
- Most studied of the three approaches and currently seen as the most promising approach for most types of spills
- No indication that fertilizer use causes algal blooms or other significant adverse impacts
- In Alaska tests, fertilizer use appeared to increase biodegradation rate by at least a factor of two.

Seeding:
- Intended to take advantage of the properties of the most efficient species of oil degrading microorganisms
- Results of field tests of seeding have thus far been inconclusive
- May not be necessary at most sites because there are few locales where oil-degrading microbes do not exist.
- Requirements for successful seeding more demanding than those for nutrient enrichment
- In some cases, seeding may help biodegradation get started faster

Use *of genetlcally engineered mlcroorganisms:*
- Probably not needed in most cases because of wide availability of naturally occurring microbes
- Potential use for components of petroleum not degradable by naturally occurring microorganisms
- Development and use could face major regulatory hurdles

SOURCE: Office of Technology Assessment, 1991.

Nutrient Enrichment

Of all the factors that potentially limit the rate of petroleum biodegradation in marine environments, lack of an adequate supply of nutrients, such as nitrogen and phosphorus, is probably the most important and perhaps the most easily modified. Nutrient enrichment (sometimes called nutrition) also has been more thoroughly studied than the other two approaches, especially now that EPA, Exxon, and the State of Alaska have carried out extensive nutrient enrichment testing on beaches polluted by oil from the *Exxon Valdez. In* part for these reasons, many scientists currently view nutrient enrichment as the most promising of the three approaches for those oil spill situations in which bioremediation could be appropriate.[66]

This approach involves the addition of those nutrients that limit biodegradation rates (but not any additional microorganisms) to a spill site and conceptually is not much different than fertilizing a lawn. The rationale behind the approach is that oil-degrading microorganisms are usually plentiful in marine environments and well adapted to resisting local environmental stresses. However, when oil is released in large quantities, microorganisms are limited in their ability to degrade petroleum by the lack of sufficient nutrients. The addition of nitrogen, phosphorus, and other nutrients is intended to overcome these deficits and allow petroleum biodegradation to proceed at the optimal rate. Experiments dating to at least 1973 have demonstrated the potential of this approach. Researchers, for example, have tested nutrient enrichment in nearshore areas

[65] Ibid., p. 15.

[66] See, for example, Lee and Levy, op. cit., footnote 66, pp. 228-234.

off the coast of New Jersey, in Prudhoe Bay, and in several ponds near Barrow, Alaska. In each case, the addition of fertilizer was found to stimulate biodegradation by naturally occurring microbial populations.[67]

The recent nutrient enrichment experiments in Alaska provided a wealth of experimental data about bioremediation in an open environment (box B). Since previous research findings had already demonstrated the general value of this approach, the experiments were intended to determine for *one type of environment* how much enhancement of natural biodegradation could be expected and to evaluate the most effective methods of application. The results provided additional evidence that application of nutrients could significantly enhance the natural rate of biodegradation on and below the surface of some beaches. As a result, Exxon was authorized by the Coast Guard on-scene coordinator, in concurrence with the Alaska Regional Response Team,[68] to apply fertilizers to the oiled beaches in Prince William Sound. To date, about 110 miles of shoreline have been treated with nutrients, and a monitoring program has been established.

Without additional research, however, it is premature to conclude that nutrient enrichment will be effective under all conditions or that it will always be more effective than other bioremediation approaches, other oil spill response technologies, or merely the operation of natural processes. The results of the Alaska experiments were influenced by the beach characteristics (mostly rocky beaches, well-washed by wave and tide action), the water temperature (cold), the kind of oil (Prudhoe Bay crude), and the type and quantity of indigenous microorganisms in Prince William Sound.

Few detailed analyses or performance data are yet available for different sets of circumstances. One smaller-scale test using the same fertilizer as in Alaska was recently conducted on beaches in Madeira polluted by the Spanish tanker *Aragon*. Results in this very different setting and with a different type of oil were not especially encouraging. Researchers speculated that the unsatisfactory results could have been due to differences in the type of oil, the concentration of fertilizer used, the lower initial bacterial activity, and/or different climatic conditions.[69] At the same time, Exxon recently used what it learned in Alaska to help degrade subsurface no. 2 heating oil spilled in a wildlife refuge bordering the Arthur Kill at Prall's Island, New Jersey. An innovative aspect of this application was the use of two trenches parallel to the beach in which to distribute fertilizer. Nutrients were dissolved with the incoming tide and pulled down the beach with the ebb tide, enabling a more even distribution than point sources of fertilizer. Exxon reports that 3 months after applying fertilizers, the oil in the treated zone had been reduced substantially relative to that in an untreated control zone.[70]

Seeding With Naturally Occurring Microorganisms

Seeding (also called inoculation) is the addition of microorganisms to a polluted environment to promote increased rates of biodegradation. The inoculum maybe a blend of nonindigenous microbes from various polluted environments, specially selected and cultivated for their oil-degrading characteristics, or it may be a mix of oil-degrading microbes selected from the site to be remediated and mass-cultured in the laboratory or in on-site bioreactors. Nutrients would usually also accompany the seed culture.

The rationale for adding microorganisms to a spill site is that indigenous microbial populations may not include the diversity or density of oil-degraders needed to efficiently degrade the many components of a spill. Some companies that advocate seeding with microorganisms also claim that commercial bacterial blends can be custom-tailored for different types of oil in advance of a spill, that the nutritional needs and limitations of seed cultures are well understood, that microbes can easily be produced in large quantities for emergency situations, and that

67 R.M. Atlas, "bioremediation of Fossil Fuel Contaminated Sites," in press, proceedings of Battelle conference on *In Situ and On-Site Bioreclamation*, March 1991. Atlas and his colleagues did some of this early work.

68 The most important members of the Alaska Regional Response Team are the U.S. Environmental Protection Agency, the Alaska Department of Environmental Conservation the U.S. Department of the Interior, the National Oceanic and Atmospheric Administration and the U.S. Forest Service.

69 M. Biscoito and M. Moreira, Museu Municipal do Funchal, Madeira, "Application of Inipol EAP22 in Porto Santo," report, July 1990.

70 P.C. Madden, Exxon Research and Engineering Co., letter and accompanying summary report on Prall's Island bioremediation to U.S. Coast Guard, Mar. 12, 1991.

Box B—The Alaska bioremediation Experiments

Following the March 1989 ***Exxon Valdez*** oil spill, the U.S. Environmental Protection Agency (EPA), Exxon, and Alaska's Department of Environmental Conservation (ADEC) undertook what is perhaps the largest and most comprehensive series of experiments on oil spill bioremediation to date. The principal objectives of the research initiated in May 1989 were to determine if the addition of nutrients to Alaska's polluted beaches would enhance the rate and extent of oil biodegradation sufficiently to support widespread use of this technology there and to evaluate which application methods could be most effective.[1] Research begun in the summer of 1990 was designed to evaluate the effectiveness and safety of several microbial products in cleaning Alaska's beaches.

The Alaska bioremediation work consisted of several discrete elements, including: 1) work begun shortly after the spill to determine if nutrient enhancement could be an appropriate technology for mitigating oil pollution of Prince William Sound's beaches; 2) application by Exxon of fertilizers to about 110 miles of polluted beaches, after the initial studies suggested that fertilizers could be both effective and safe; 3) additional EPA studies to support Exxon's treatment program and further evaluate application techniques; 4) a long-term program to monitor treated beaches, conducted jointly by EPA, Exxon, and ADEC; and 5) evaluation of the potential of adding microbes to Alaska's beaches to stimulate biodegradation.

Several types of nutrients and application methods were evaluated, including slow-release, water soluble fertilizers in both briquette and granular forms; a water soluble fertilizer applied with a sprinkler system; and a liquid oleophilic fertilizer,[2] applied with sprayers, specially formulated to keep nutrients and oil in contact. Visual changes between control and experimental plots were observed, and changes in oil chemistry, microbial populations, and oil weight over time were measured. Findings pertain specifically to Prince William Sound, which has a number of features favorable to nutrient enrichment: a high percentage of naturally occurring hydrocarbon-degrading bacteria, low concentrations of ammonia and phosphate in seawater, highly porous beaches, and large tidal fluxes. Although the work carried out in Alaska is important to bioremediation research and applications in other areas, the same results cannot be expected elsewhere. The major findings follow:

- Based on a synthesis of all available evidence, researchers concluded that biodegradation on beach surfaces was accelerated as much as two- to four-fold by a single application of fertilizer; thus, the addition of nutrients to Alaska's beaches did significantly stimulate the rate of biodegradation. The water soluble fertilizer delivered by a sprinkler system proved the most effective approach, but this method was impractical on a large scale. The oleophilic fertilizer and slow-release granules were almost as effective and more practical to use. EPA determined that the most practical approach for this setting was to apply the oleophilic fertilizer to beaches with surface oiling and to use both oleophilic fertilizer and fertilizer granules where surface and subsurface oil were found.
- After several weeks, dramatic visual changes were observed in the amount of oil on beaches treated with fertilizer. Visual changes do not provide quantitative data or prove that biodegradation is occurring. However, similar changes observed in beaches treated with the "plain" water soluble fertilizer and those treated with oleophilic fertilizer provided evidence that visually cleaner beaches

Photo credit: Environmental Protection Agency

Visual effect of oleophilic fertilizer on the biodegradation of surface oil in Snug Harbor, Alaska. The clear "window" indicates where the fertilizer was applied.

[1] U.S. Environmental protection Agency, office of Research and Development, "Interim Report: Oil Spill bioremediation Project," Feb. 28, 1990, 220 pp. See also P.H. Pritchard and C.F. Costa, "EPA Alaska Oil Spill Bioremediation Project," *Environmental Science and Technology,* March 1991, pp. 372-379. Much of the material in this box was reported in these two citations.

[2] For more on oleophilic fertilizers see A. Ladousse and B. Tramier, Societe Nationale ELF AQUITAINE, "Results of 12 Years of Research in Spilled Oil bioremediation: Inipol EAP 22," 1990.

Continued on next page

Box B—The Alaska bioremediation Experiments-Continued

resulted directly from the addition of nutrients, not from the suggested rock washing effects of oleophilic fertilizers. The oleophilic fertilizers evidently worked as intended, sequestering nutrients at the oil-water interface where microorganisms could be effective.

- Changes in oil chemistry provided additional evidence that biodegradation was limited by lack of nutrients. Analysis of fertilizer-treated samples and samples that had been artificially weathered to control for evaporation indicated that many of the easily degradable constituents of petroleum in fertilizer-treated samples had decreased substantially over 4 weeks. Some harder-to-degrade fractions of oil also appeared to decrease. No conclusions could be drawn about other difficult-to-degrade fractions because good measurement methods had not been devised for them.
- In early testing, statistically significant increases in the oil degrading microbial population-increases that would correlate strongly with the rate of biodegradation-were not observed because of the high variability in numbers of bacteria in each sample. However, later results from the joint monitoring program appeared to indicate a sustained three- to four-fold increase in microbial activity.
- No statistically significant conclusions could be made about the rate of biodegradation from "before and after" measurements of the weight of oil samples. Although a significant decrease in oil residue weight over time would be evidence of degradation, this might not be a good criterion because bacterial production of high-molecular-weight compounds can occur. Precise measurements were impossible because oil was distributed unevenly on the beaches. Although the rate of biodegradation ***was probably*** the same in all areas, samples from more heavily oiled areas indicated a slower rate of biodegradation than samples from lightly oiled areas.
- The monitoring program indicated that enhanced microbial activity could be sustained for more than 30 days from a single fertilizer application. Additional applications were found to increase microbial activity. In particular, a second application of fertilizer after 3 to 5 weeks replenished nutrients and stimulated microbial activity five- to ten-fold.[3]
- Fertilizer applications appeared to enhance biodegradation to a depth of at least 50 centimeters on treated beaches. Researchers found increased nitrogen nutrients, sufficient dissolved oxygen, and increased microbial numbers and activity at this depth following treatment.[4]
- Although evidence was not conclusive, researchers suspected that primary treatment with mechanical methods resulted in a more even distribution of oil on the beaches and hence prepared the beaches for optimum bioremediation.[5]
- Results of the 1990 research on two microbial products were inconclusive, with no statistically significant enhancement of the rate of biodegradation over natural rates. However, the tests were conducted on oil that had weathered and degraded naturally during the 18 months since the ***Exxon Valdez*** spill. The more easily degraded components of the oil had already disappeared. The limited testing period-27 days-may also have affected the results.[6]

[3]R. Prince, J. Clark, and J. Lindstrom, "bioremediation *Monitoring Program,*" December 1990, pp. 2,85. The authors of this report represent Exxon, EPA, and the Alaska Department of Environmental Conservation respectively.

[4]Ibid.

[5]Pritchard and Costa, op. cit., footnote 1.

[6]A. Venosa, EPA, Risk Reduction Engineering Laboratory, presentation of research results at EPA-sponsored bioremediation meeting, Las Vegas, NV, Feb. 20, 1991.

seed cultures can be stored, ready for use, for up to 3 years.[71]

The value of introducing nonindigenous microorganisms to marine environments is still being evaluated. With some exceptions, the scientific community has not been encouraging about the promise of seeding marine oil spills. Controlled studies have not been conducted in such settings, so no data are available to evaluate the effectiveness of this approach. Many scientists question the necessity of adding microbes to a spill site because most locales have sufficient indigenous oil-degrading microbes, and in most environments biodegradation is limited more by lack of nutrients than by lack of

[71]Applied Biotreatment Association, op. cit., footnote 46, pp. 13-14.

microbes.[72] At many spill sites, a very low level of oil is often present as ''chronic" input, inducing oil-degrading capability in naturally occurring microorganisms. Moreover, the requirements for successful seeding are more demanding than those for nutrient enrichment. Not only would introduced microbes have to degrade petroleum hydrocarbons better than indigenous microbes, they would also have to compete for survival against a mixed population of indigenous organisms well adapted to their environment. They would have to cope with physical conditions (such as local water temperature, chemistry, and salinity) and predation by other species, factors to which the native organisms are likely to be well adapted.

The time required for introduced microbes to begin metabolizing hydrocarbons is also important. If a seed culture can stimulate the rapid onset of biodegradation, it would have an advantage over relying on indigenous microbes that may take time to adapt. Despite some claims, seed cultures have not yet demonstrated such an advantage over indigenous microbial communities. Seed cultures are typically freeze-dried (and therefore dormant) and require time before they become active.[73] Seed cultures also must be genetically stable, must not be pathogenic, and must not produce toxic metabelites.[74]

Some laboratory and small-scale experiments in controlled environments have demonstrated that seeding can promote biodegradation.[75] However, it is exceedingly difficult to extrapolate the results of such tests to open water where many more variables enter the picture. Results of experimental seeding of oil spills in the field have thus far been inconclusive.[76] As noted in box B, recent EPA tests of two commercial products applied to contaminated beaches in Alaska concluded that, during the period of testing, there was no advantage from their use.[77] In a well-publicized attempt to demonstrate seeding at sea, one company applied microorganisms to oil from the 1990 *Mega Borg* spill in the Gulf of Mexico.[78] Although the experiment aroused some interest, the results were inconclusive and illustrated the difficulty of conducting a controlled bioremediation experiment at sea and measuring the results. Although there were changes observed in the seeded oil, in the absence of controls the experiment could not tell whether they were due to biodegradation or bioemulsification (the process in which microbes assist the dispersal of surface oil), or were unrelated to the seeding. (Even if bioemulstilcation rather than biodegradation was the process at work in this experiment, it may be of potential interest for oil spill response and could be investigated further.)

Photo credit: National Oceanic and Atmospheric Administration

Application of a microbial product to Marrow Marsh in response to an oil spill caused by the collision of the Greek tanker Shinoussa with three barges in the Houston Ship Channel.

An attempt has been made to apply a seed culture to a polluted salt marsh. In July 1990 the Greek tanker *Shinoussa* collided with three barges in the Houston Ship Channel, resulting in a spill of about 700,000 gallons of catalytic feed stock, a partially refined oil. Some of this oil impacted neighboring Marrow Marsh. Microbes were applied to experimental areas within the marsh, and control areas were established. Visual observations made by the scientific support coordinator who monitored the

[72] Lee and Levy, op. cit., footnote 22, p. 229; *see also* Atlas, op. cit., footnote 23, p. 218.

[73] R.M. Atlas, Department of Biology, University of Louisville, personal communication, Nov. 29, 1990.

[74] R.M. Atlas, ''Stimulated Petroleum Biodegradation,'' *Critical Reviews of Microbiology,* vol. 5, 1977, pp. 371-386.

[75] See, for example, Texas General Land Office, "Combating Oil Spills Along the Texas Coast: A Report on the Effects of Bioremediation,'' June 12, 1990; see also Leahy and Colwell, op. cit., footnote 28, p. 311.

[76] P.H. Pritchard and C.F. Costa, ''EPA Alaska Oil Spill bioremediation Project,'' *Environmental Science and Technology,* March 1991, pp. 372-379.

[77] E. Berkey, National Environment Technology Applications Corp., personal communication, Feb. 15, 1991.

[78] Texas General Land *office, Mega Borg Oil Spill: An Open Water bioremediation Test,* July 12, 1990.

application for the National Oceanic and Atmospheric Administration (NOAA) indicated that treated oil changed color within a few minutes to a few hours after treatment, but that after several days there were no significant visual differences between treated and untreated plots. More importantly, chemical analyses indicated "no apparent chemical differences in petroleum hydrocarbon patterns between treated and untreated plots several days after treatment. '[79] Not all of the monitoring data have been analyzed yet, so a final determination of effectiveness has not been made.

Seed cultures may be most appropriate for situations in which native organisms are either present as slow growers or unable to degrade a particular hydrocarbon. Especially difficult-to-degrade petroleum components, such as polynuclear aromatic hydrocarbons, might be appropriate candidates for seeding.[80] In other cases, if a time advantage can be realized, there may be some utility in seeding with a culture consisting of indigenous organisms.[81] Thus, the potential environmental adaptation problems of nonindigenous organisms might be avoided. In many cases, fertilizers would also have to be "added.

Seeding may offer promise in environments where conditions can be more or less controlled. In such cases one would have to consider the proper choice of bacteria, a suitable method of application, and suitable site engineering. Arrangements would have to be made for keeping cells moist and in contact with the oil; for protecting them from excess ultraviolet light; for providing adequate nutrients; and for controlling temperature, pH, and salinity. However, before claims about the utility of seeding marine oil spills can be proved (or disproved), additional research-verified by repeatable experiments—is required.

Seeding With Genetically Engineered Microorganisms

Although it was not demonstrably superior to indigenous organisms and has never been tested in the field, the frost organism ever patented was a microorganism genetically engineered to degrade oil.[82] The rationale for creating such organisms is that they might possibly be designed either to be more efficient than naturally occurring species or to have the ability to degrade fractions of petroleum not degradable by naturally occurring species. To be effective, such microorganisms would have to overcome all of the problems related to seeding a spill with nonindigenous microbes.

EPA has not yet conducted any GEM product reviews for commercial applications, although at least two companies are considering using genetically engineered products for remediating hazardous waste. Since the development and use of GEMs are still limited by scientific, economic, regulatory, and public perception obstacles, the imminent use of bioengineered microorganisms for environmental cleanup is unlikely. Lack of a strong research infrastructure, the predominance of small companies in the bioremediation field, lack of data sharing, and regulatory hurdles are all barriers to the commercial use of genetically engineered organisms.[83] The development of GEMs for application to marine oil spills does not have high priority. Many individuals, including EPA officials, believe that we are so far away from realizing the potential of naturally occurring microorganisms to degrade marine oil spills that the increased problems associated with GEMs render them unnecessary at this time.[84]

ENVIRONMENTAL AND HEALTH ISSUES

To date, no significant environmental or health problems have been associated with the testing or application of bioremediation technologies to ma-

[79] A.J. Mearns, Leader, BioAssessment Team, National Oceanic and Atmospheric Administration, "Observations of An Oil Spill bioremediation Activity in Galveston Bay, Texas,'' March, 1991.

[80] Atlas, "bioremediation of Fossil Fuel Contaminated Sites," op. cit., footnote 67.

[81] R. Colwell, OTA bioremediation Workshop, Dec. 4, 1990.

[82] See, for example, D.A. Friello, J.R. Mylroie, and J.M. Chakrabarty, ''Use of Genetically Engineered Multiplasmid Microorganisms for Rapid Degradation of Fuel Hydrocarbons,"*Biodeterioration of Materials, No. 3, 1976,* pp. 205-214.

[83] U.S. Congress, Office of Technology Assessment, *Biotechnology in a Global Economy* (Washington, DC: U.S. Government Printing Office, scheduled for publication sumner 1991).

[84] A.W. Lindsey, Director, Office of Environment Engineering and Technology, U.S. Environment@ Protection Agency, personal communication, September 1990.

rine oil spills. Experience with bioremediation in marine settings is still limited, so it is premature to conclude that its use will always be safe or that possible risks will be acceptable in all of the circumstances in which bioremediation might be employed. The evidence to date, nevertheless, suggests that risks will be unimportant in most situations.

Concerns have been raised about ***several potential*** adverse environmental effects. Among these are the possibility that the addition of fertilizers could cause eutrophication, leading to algal blooms and oxygen depletion; that components of some fertilizers could be toxic to sensitive marine species or harmful to human health; that the introduction of nonnative microorganisms could be pathogenic to some indigenous species; that the use of bioremediation technologies could upset ecological balances; and that some intermediate products of bioremediation could be harmful.

The possible adverse effects of nutrient enrichment were examined in some detail during the 1989-90 Alaska beach bioremediation experiments.[85] To determine the potential for eutrophication in Prince William Sound, researchers measured ammonia, phosphate, chlorophyll, bacterial numbers, and primary productivity in the water column directly offshore of fertilizer-treated beaches and in control areas. They could find no significant difference between measurements in control areas and those in experimental areas.[86] There were no indications that fertilizer application stimulated algal blooms.

The possible toxicity of fertilizer components was examined in both laboratory and field tests on a number of marine species, including sticklebacks fish, Pacific herring, silver salmon, mussels, oysters, shrimp, and mysids. In the absence of tidal dilution, certain components of the oleophilic fertilizer were mildly toxic to oyster larvae, the most sensitive marine species.[87] However, in the view of researchers working in Alaska, such effects were transient and limited to areas immediately adjacent to fertilized shorelines.[88] The concentration of ammonia, the only component of fertilizers shown to be acutely toxic to marine animals, never reached toxic levels.

Photo credit: Exxon Corp.

Wildlife deterrent device used to keep animals off Alaska beaches sprayed with oleophilic fertilizer. Such devices were used during the short period after application in which the butoxyethanol component of the fertilizer could be harmful to animals coming into contact with it.

The butoxyethanol constituent of the oleophilic fertilizer is potentially harmful to some mammals. This constituent, however, evaporated from beach surfaces in less than 24 hours, during which time wildlife deterrent devices were employed. Care had to be taken, as well, by humans applying the oleophilic fertilizer to avoid inhalation or skin contact. Researchers were also able to show that the oil itself did not wash off the treated beaches and

[85] See, for example, U.S. Environmental Protection Agency, Office of Research and Development *Interim Report: Oil Spill bioremediation Project*, Feb. 28, 1990; R.C. Prince, J.R. Clark, and J.E. Lindstrom, *bioremediation Monitoring Program*, December 1990; and P.H. Pritchard and C.F. Costa, "EPA Alaska Oil Spill bioremediation Project,'' *Environmental Science and Technology*, March 1991.

[86] Ibid.

[87] U.S. Environmental Protection Agency, "Alaskan Oil bioremediation Project: Update," EPA/600/13-89/073, July 1990, p. 11.

[88] Prince et al., op. cit., footnote 85, p. 72.

accumulate in the tissues of marine test species. In this environment, dilution, tidal mixing, and evaporation reduced the potential for significant impacts. In some low-energy environments (e.g., protected bays), greater impacts might occur. In other environments, the species present, water depth, and water temperature are all variables to consider in estimating potential impact. The effect of any impact of treatment, however, must be considered in view of the damage already caused by oil.

Evidence is also lacking that introduced organisms might be pathogenic to other life forms. In a series of experiments with North Slope crude oil, for example, researchers failed to find any significantly greater invertebrate mortality with bacterial seeding (or fertilization) than occurred with crude oil alone.[89] However, microorganisms to be considered as seeding candidates must be screened carefully to eliminate potential human or animal pathogens, including opportunistic pathogens such as *Pseudomonas* spp.

The possibility that introduced microbes might proliferate and upset the ecological balance appears to be of less concern. If effective at all, such organisms should die and be preyed on by protozoa once they have utilized the oil from a spill.[90] Of greater concern is that microbes introduced from other environments will not be able to compete as well as native species and will die ***before they can*** do their job effectively. EPA's Office of Pesticides and Toxic Substances has been developing procedures for evaluating the toxicity of biotechnology products. In concert with EPA's Office of Research and Development, it is establishing tests to evaluate the potential pathogenicity of nonindigenous microbes.[91]

Similar but greater concerns attend the introduction of genetically engineered organisms. Before such organisms are likely to be introduced to the marine environment (if they have a role to play at all), more basic knowledge of their potential impacts on that environment will be required, and regulatory officials and the public will have to become more familiar with biological mitigation techniques.

An additional concern is that although bacteria may break down the complex hydrocarbons contained in oil, they could leave behind products of partial biodegradation that are more toxic to marine life than the original constituents of the oil.[92] However, in time, intermediate products of possible concern, such as quinones and naphthalenes, are likely to be broken down further and thus unlikely to accumulate in the environment.[93]

bioremediation IN RELATION TO OTHER RESPONSE TECHNOLOGIES

Whether bioremediation technologies will be considered for use as primary or secondary response tools, or will be deemed of no use at all, will depend on the circumstances of each oil spill. All response technologies have the common purpose of minimizing the damage caused by a spill. How well a technology can accomplish this goal indicates its effectiveness. The perfect response technology has not been developed, and numerous uncontrollable variables may reduce effectiveness far below what it would be under optimal circumstances. As the size of a spill increases, for example, the difficulty of responding to it by any means grows. Adverse sea and weather conditions may greatly reduce the effectiveness of any open sea response. Even technologies that are adequate in some spill situations will be much less effective if they cannot be deployed, operated, and maintained easily.

Before bioremediation is likely to be considered as a response tool, it must be deemed not only effective for its intended use, but also more effective than traditional technologies. The effectiveness and safety of bioremediation technologies for responding to different types of spills have not yet been established adequately, and on-scene coordinators and other decisionmakers generally are not familiar with these technologies. Hence, most decisionmakers are reluctant to try bioremediation if other techniques could be effective. More traditional methods are preferred, and experimentation during

[89] R.M. Atlas and M. Busdosh, "Microbial Degradation of Petroleum in the Arctic," *Proceedings of the 3rd International Biodegradation Symposium*, Kingston, RI, August 1975, p. 85. The seeding trials used an organism from the environment being treated.

[90] R. Colwell, Maryland Biotechnology Institute, University of Maryland, personal communication, February 1991.

[91] U.S. Environmental Protection Agency, Office of Research and Development, bioremediation Action Committee, "Summary of June 20, 1990 Meeting," July 1990.

[92] M.C. Kennicutt, Geochemical and Environmental Research Group, Texas A&M University, personal communication, October 1990.

[93] R.M. Atlas, Department of Biology, University of Louisville, personal Communication February 1991.

a real spill is not the best strategy. The acceptability (or nonacceptability) of bioremediation technologies may take time to establish. The example of dispersant development may provide a relevant analogy for bioremediation in the immediate future: Dispersants have been advocated by many and considered for more widespread use for years, but uncertainty about their effectiveness, as well as continuing environmental concerns and regulatory limitations, has considerably slowed their acceptance in the United States. Probably the most serious setback was the fact that a few early dispersants were toxic; however, since the frost attempts to formulate successful dispersants, toxicity has been addressed and, in some cases, is no longer a serious factor.

bioremediation technologies have been considered for use at sea, on beaches, and in especially sensitive habitats such as salt marshes. It is unclear whether bioremediation could be useful on the open ocean. Most of the scientific community and many oil spill professionals remain skeptical about the utility of bioremediation at sea because rigorously controlled and documented experiments have not yet been done. Several companies have advocated using bioremediation for open ocean spills, but they have not yet produced convincing evidence that their products work as claimed.

In addition to the previously noted problems associated with seeding, a potentially significant problem at sea may be the difficulty of keeping microbes or nutrients in contact with spilled oil long enough to stimulate degradation.[94] This is afar more difficult task than on land or beaches because wind, waves, and currents create a dynamic, changeable environment. As with dispersants, efficient application could also be difficult because ocean conditions are often less than ideal, and the oil may be difficult to locate, may be emulsified or broken into windrows, or in a major spill, may have spread over many square miles. These same problems also limit the effective use of booms and skimmers.

The probability is low that a response with *any type* of technology would be mounted for a spill far out to sea that does not threaten the coast. The initial goal in responding to spills that do threaten the coast is to prevent oil from reaching the shore. Thus, unless seeding, nutrient enrichment, or both can be shown to act quickly *and can be* applied efficiently, spill fighters typically would prefer to get the oil out of the water as quickly as possible or to disperse it. If open sea bioremediation can be shown to be effective over a longer period (i.e., weeks), it might be useful as a response technology either after a full-scale mechanical or dispersant effort had been launched or if such an effort was not possible. Conceivably, nutrients or a seed culture might also be applied to the oil and its residues remaining in the water after the intentional (or unintentional) burning of oil. bioremediation is less likely to be attempted following the use of chemical dispersants; however, there is evidence that some microorganisms may stimulate bioemulsification and thus cause oil to disperse upon application. This possibility and the possible merits and limitations associated with it have not been investigated thoroughly.

Although bioremediation at sea has not been convincingly demonstrated to be effective in *any type* of spill situation, alternative response technologies leave much to be desired. bioremediation, although unproven, appears relatively promising to some. The State of Texas, for example, has been particularly enthusiastic about its potential. Texas has taken the position that there is little to lose by trying it and, given the limitations of other technologies, potentially much to gain or, at least, to learn.[95] The Texas State Water Commission points out that those responsible for responding to an emergency may not be able to wait for a definitive unambiguous ruling from the scientific community on the effectiveness of bioremediation. The Federal Government and other States have been more cautious. The State of Alaska, for example, has tentatively concluded that bioremediation would not be appropriate as an emergency response tool for nearshore spills threatening the coast.[96] In any case, controlled testing, difficult to conduct on the open ocean, will be required to evaluate the potential of bioremediation at sea. ~~bioremediation shows promise for at least some types of open water spills, effective application techniques would still have to be developed for the promise of the technology to be fulfilled.

[94] R.M. Atlas, Department of Biology, University of Louisville, personal communication, Nov. 29, 1990.

[95] B.J. Wynne, Chairman, Texas Water Commission statement at OTA Workshop on bioremediation, Dec. 4, 1990.

[96] E. Piper, Alaska State On-Scene Coordinator, *Exxon Valdez Oil* Spill Response. Letter to Texas Water Commission, Oct. 19, 1990.

Somewhat more is known about the potential for bioremediation of beaches, thanks largely to the Alaska experiments. The potential use of bioremediation methods on at least some beaches looks promising, but, as previously noted, results from the Alaska experiments cannot be extrapolated in toto to other types of beaches or spill situations (especially without more precise knowledge of the effect of environmental and microbiological variables on the rate and extent of biodegradation).

The greater promise of bioremediation for oiled beaches is due in part to the fact that bioremediation is more easily controlled and monitored onshore than it is at sea. Application of nutrients or seed cultures is also easier and less subject to disruption by adverse conditions. Also, once oil reaches a beach, there is usually more time to consider the approach to take: certain damage has already been done, and the emergency response required to deal with oil seeping from a stricken tanker, for example, is no longer quite so necessary.

Depending on circumstances, bioremediation of beaches may be appropriate sometimes as a primary response tool and sometimes as a secondary tool. An important consideration is how heavily oiled a beach is. Heavily oiled beaches may require removal of gross amounts of oil by mechanical means before bioremediation can be a practical finishing tool. Some lightly oiled beaches may not require any treatment. Moderately oiled beaches are likely the main candidates for primary bioremediation treatment. After reviewing the Alaska bioremediation experiments, the Alaska Department of Environmental Conservation concluded that bioremediation was useful as a finishing or polishing tool, but that pooled oil, tar balls, mousse, asphalt, and other heavy concentrations of oil should be picked up with conventional manual and mechanical techniques.[97]

The effectiveness of bioremediation on beaches may also depend on the coarseness of the beach, but the relationship between the size of oiled sediments and the rate of biodegradation has not been evaluated thoroughly. The beaches treated in Alaska with some success were all very coarse, consisting mostly of cobbles and coarse sand. It is uncertain how successful bioremediation of freer grained beaches will be, especially when oil is trapped below the surface. In very fine-grained sediments, lack of oxygen below the surface may limit the rate of biodegradation.

bioremediation, where effective, may offer a promising option for beach cleanup because the existing mechanical technologies can cause additional damage to beaches and beach biota.[98] This damage may be unavoidable if the goal is to "restore" a beach as quickly as possible. Doing nothing (i.e., letting the beach recover naturally at a slower rate) may sometimes be preferable to using mechanical technologies, but this is seldom politically acceptable. bioremediation offers the possibilities of being faster than simply allowing nature to take its course unassisted and of avoiding the negative impacts of mechanical technologies. Moreover, when beaches are inaccessible, the mechanical equipment that can be brought into use may be limited, but fertilizers or seed cultures can be dispensed without the need for massive machinery. In general, bioremediation is less costly and less equipment- and labor-intensive than mechanical cleanup technologies, which suggests a clear advantage for bioremediation where it can be used effectively *instead* of other technologies (e.g., moderately oiled beaches). The advantage is also evident where it can be used as a secondary technology (i.e., as a finishing tool), because it offers the possibility of a more complete solution, more quickly attained (however, the total cost of the cleanup may be greater).

Salt marshes and other sensitive environments, even more than beaches, may be further damaged by intrusive mechanical technologies. For these environments, bioremediation could be the only feasible alternative to doing nothing. Little work has been done in these settings to evaluate the effectiveness or environmental impacts of using bioremediation. Biodegradation of oil stranded in salt marshes is generally limited by oxygen availability. However, the results of one recent study of *waxy crude oils*[99] *in* salt marshes suggest that nutrient enrichment may be an effective countermeasure, provided that large

[97] Ibid.

[98] M.S. Foster, J.A. Tarpley, and S.L. Dearn, "To Clean or Not To Clean: The Rationale, Methods, and Consequences of Removing Oil From Temperate Shores," *The Northwest Environmental Journal*, vol. 6, 1990, pp. 105-120.

[99] Waxy crude oils tend to spread, evaporate, and naturally disperse very slowly on water, and usually survive in a relatively fresh state considerably longer than conventional oils.

amounts of oil do not penetrate beneath the aerobic surface layer. Where oil did penetrate the surface layer, the researchers observed little degradation.[100]

Even bioremediation activities, if they are not carefully conducted, have the potential for being intrusive in salt marshes. The scientific support coordinator of the National Oceanic and Atmospheric Administration who monitored the application of microorganisms to Marrow Marsh in the Houston Ship Channel noted, for example, that excessive foot traffic associated with bioremediation operations caused some unnecessary damage to marsh grass.[101]

bioremediation ACTIVITIES IN THE PUBLIC AND PRIVATE SECTORS

The Environmental Protection Agency

EPA is the lead Federal agency for oil spill bioremediation research. Both the Department of Energy and the Department of Defense are actively engaged in research on bioremediation of hazardous waste, but neither these nor other Federal agencies are engaged in research on bioremediation of marine oil spills. EPA regards biotechnologies as having significant potential for the prevention, reduction, and treatment of pollution, and the Agency has placed considerable emphasis on the demonstration and development of these technologies.[102] This coincides with an important general EPA goal of promoting the development of new and innovative technologies to address environmental problems. Agency activities in support of bioremediation for marine oil spills represent a small fraction of its overall biotechnology activities. Nevertheless, EPA would like to establish the technical basis for a national bioremediation response capability for oil spills.[103]

In February 1990, EPA convened a meeting of interested industry, academic, and government personnel "to prepare an agenda for action" for increasing the use of biotechnology. One important outcome of this meeting was the formation of the bioremediation Action Committee (BAC). The objective of the Committee is to facilitate the safe development and use of biotechnology as a solution to environmental problems. The BAC has now been subdivided into six subcommittees: Oil Spill Response, Treatability Protocol Development, Research, Education, Data Identification and Collection, and Pollution Prevention (figure 2). Several of these subcommittees have, in turn, been further subdivided. All subcommittees report to the assistant administrator of EPA's Office of Research and Development (ORD), who functions as the chair of the BAC. Many of the same industry, academic, and governmental representatives who attended the February meeting are participants on the BAC or its subcommittees.

The Oil Spill Response Subcommittee is concerned directly with the bioremediation of marine oil spills. Its major goals are: 1) to evaluate scientific and applied engineering data on the safety and effectiveness of bioremediation technologies; 2) to assess the information required for bioremediation decisionmaking by Federal on-scene coordinators and State oil spill response officials; 3) to prepare interim guidelines on when and how to use bioremediation technologies; and 4) to investigate longer-term issues for incorporating bioremediation into the National Spill Response Plan.[104] An eventual result of deliberations relating to these goals could be the design of a national bioremediation oil spill response plan.[105] However, further research and development of more reliable technologies are required before EPA is likely to undertake the effort required to develop such a plan. In the meantime, the Subcommittee has prepared interim guidelines to assist Regional Response Teams in assessing the desirabil-

[100] K. Lee and E.M. Levy, "Bioremediation: Waxy Crude Oils Stranded on Low-Energy Shorelines," *Proceedings: 1991 Oil Spill Conference, San Diego, CA*, Mar. 4-7, 1991.

101 M-, op. cit., footnote 79.

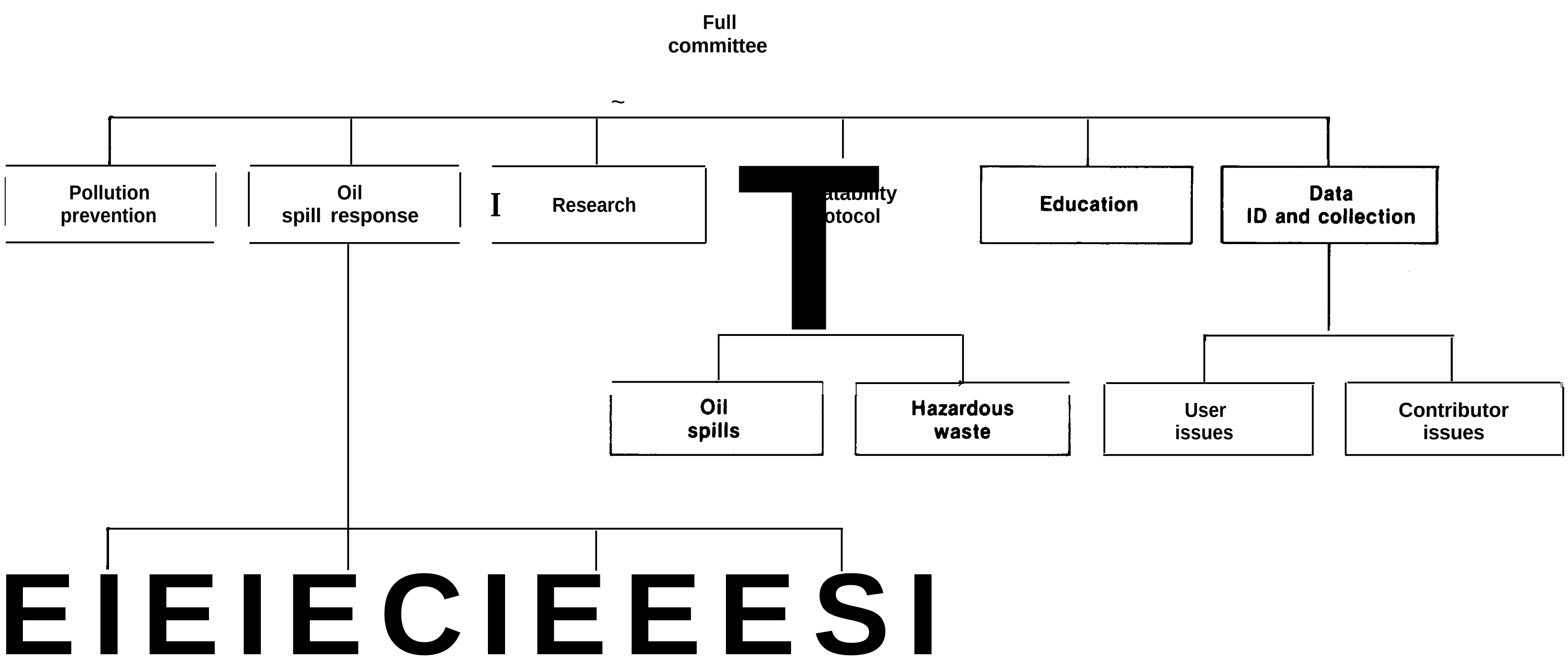
[102] U.S. Environmental Protection Agency, "Summary Report on the EPA-Industry Meeting on Environmental Applications of Biotechnology," Crystal City, VA, Feb. 22, 1990.

[103] U.S. Environmental Protection Agency, bioremediation Action Committee, "Summary of June 20, 1990 Meeting," p. 3.

[104] U.S. Environmental Protection Agency, Office of Research and Development, Bioremediation Action Committee, "Summary of November 7, 1990 Meeting," Washington, DC, Nov. 7, 1990, pp. 4-7.

[105] F.M. Gregorio, member, *Oil* Spill Response Subcommittee, "Development of a National bioremediation Spill Response Plan," Aug. 27, 1990.

Figure 2--Organization of the bioremediation Action Committee



SOURCE: U.S. Environmental Protection Agency.

ity of bioremediation and in planning for its use.[106] Such guidelines will enable decisionmakers to make quick and defensible decisions about the use of bioremediation technologies.

The Treatability Protocol Development Subcommittee has focused its attention on providing technical advice on the development of protocols for testing the applicability and effectiveness of bioremediation technologies in different environmental settings. Protocols use both chemical analyses and bioassays to evaluate a bioremediation product's ability to degrade a waste product or pollutant and to ensure that the product is safe to introduce into the environment.[107] Performance criteria included in the protocols can provide a standard for technology developers against which they can compare their processes.

The development of oil spill protocols is a high-priority EPA activity: without them it is not possible to validate technology or process claims made by product vendors. This activity is being carried out largely through the National Environmental Technology Applications Corp. (NETAC) and EPA's ORD labs (see below). One laboratory-based protocol has already been developed and used to evaluate products intended for use on Alaska's beaches. Work is in progress on the development of an open water protocol, as well as on protocols for sensitive marine environments (e.g., marshes). The Subcommittee will also be involved in developing protocols for bioremediation of hazardous wastes.

The Treatability Protocol Development Subcommittee is also addressing several policy issues related to protocols. For example, bioremediation companies are concerned about having products retested that did not do well initially. Another issue is the recourse available to companies that disagree with test procedures. A third is the means by which results will be reported. (Results will probably be reported as statistically superior to, not statistically different from, or statistically inferior to a standard; products are unlikely to be ranked. Also, products will be judged on different criteria, including efficacy, shelf life, toxicity, etc. A product that does well according to one criterion may not do well on another.) Finally, there is the question of who pays for product testing. EPA appears receptive to some cost sharing with product developers, but only for those products that have met minimum criteria.[108] A cost sharing program involving EPA, the petroleum industry, and product vendors might also be arranged so that a broad commercial testing program could be established.[109]

The main objective of the Research Subcommittee is to identify high priority needs for *general* bioremediation research,[110] not specifically for oil spill applications, but research advances in priority areas could directly or indirectly benefit the latter. The Education Subcommittee will evaluate future needs for scientists, technicians, and engineers in bioremediation research and applications, as well as ways to educate the public about the use of biotechnologies.

Although considerable bioremediation information has been generated by industry, States, and Federal agencies, this information is not necessarily easily accessible, nor has it been certified or standardized for easy use by others. The Data Identification and Collection Subcommittee will focus its efforts on identifying data on field applications and tests of bioremediation technologies (including marine spill applications such as those in Alaska and Texas), on providing guidance for making data available without compromising client or proprietary information, and on establishing routine procedures for submission of data. The Subcommittee has recommended that EPA's Alternative Treatment Technology Information Center (ATTIC) database be used as the central database for all biological treatment technologies. Designed primarily as a retrieval network for information on innovative technologies for treating hazardous wastes, ATTIC should have no trouble incorporating

[106] U.S. Environmental Protection Agency, Subcommittee on National bioremediation Spill Response, bioremediation Action Committee, "Interim Guidelines for Preparing bioremediation Spill Response Plans" (draft), Feb. 11, 1991.

[107] E.B. Berkey, National Environmental Technology Applications Corp., "Presentation of the Progress of the Treatability Protocol Development Subcommittee," Washington, DC, Nov. 7, 1990.

[108] Remarks of J. Skinner, Deputy Assistant Administrator of the Office of Research and Development, U.S. Environmental Protection Agency, during Nov. 7, 1990 meeting of the bioremediation Action Committee.

[109] S. Lingle, Deputy Director, Office of Environmental Engineering and Technology Demonstration, U.S. Environmental Protection Agency, personal communication Feb. 20, 1991.

[110] U.S. Environment Protection Agency, op. cit., footnote 103.

data on the applications of bioremediation for marine oil spills.[111]

The Pollution Prevention Subcommittee is the newest BAC committee. Its purpose is to provide advice to EPA on the potential of biotechnologies for preventing pollution. An example of a pollution prevention application for biotechnology already in use is the treatment of oily ballast water from ships in onshore biological treatment facilities before releasing it to the ocean. This practice is followed, for instance, in Prince William Sound.

A number of EPA laboratories have contributed to the Agency's bioremediation research effort. Prominent among these are the Environmental Research Laboratory in Gulf Breeze, Florida; the Risk Reduction Engineering Laboratory in Cincinnati, Ohio; the Environmental Research Laboratory in Athens, Georgia; and the Environmental Monitoring Systems Laboratory in Las Vegas, Nevada. Table 4 indicates some key bioremediation research needs.

Prior to the ***Exxon Valdez oil*** spill, EPA had virtually no money for oil spill research. Shortly after the March 1989 spill, EPA and Exxon signed a cooperative research and development agreement and initiated the Alaskan Oil Spill bioremediation Project (described above). During 1989, EPA redirected about $1.6 million to the project and Exxon contributed about $3 million. In 1990, Congress appropriated $1 million to EPA for oil spill research, which was applied entirely to the continuing Alaskan project. As in 1989, Exxon contributed about twice as much. To date, about $8 million has been devoted to the Alaskan project, and 1991 will probably be its last year.

Congress appropriated $4 million to EPA for oil spill research activities for fiscal year 1991. EPA expects to spend roughly $2 million of this, not including salaries, for bioremediation research. Beginning in 1992, the Oil Pollution Act of 1990 authorizes a maximum of approximately $21 million annually for oil pollution research and development. The exact amount must be approved by Congress each year, and much less could be appropriated. EPA has asked for $3.5 million of funds available through OPA for fiscal year 1992, a sizable proportion of which will likely be devoted to bioremediation research.

Table 4-Key Research Needs

- **Better understanding of environmental parameters governing the rate and extent of biodegradation in different environments**
- **Improving methods for enhancing the growth and activity of petroleum degrading bacteria**
- **Development of better analytical techniques for measuring and monitoring effectiveness**
- **Field validation of laboratory work**
- **Investigation of what can be done to degrade the more recalcitrant components of petroleum, e.g., asphaltenes**
- **Improving knowledge of the microbiology of communities of microorganisms involved in biodegradation**
- **Better understanding of the genetics of regulation of biodegradation**

SOURCE: Office of Technology Assessment, 1991.

NETAC and Commercialization of Innovative Technologies

EPA has entrusted some of the work of developing new bioremediation protocols to the National Environmental Technology Applications Corp. NETAC is a nonprofit corporation established in 1988 through a cooperative agreement between EPA and the University of Pittsburgh Trust. It was created to help commercialize innovative environmental technologies such as bioremediation.

The *Exxon Valdez* spill provided the opportunity for NETAC to become involved in evaluating bioremediation technologies. After the spill, EPA and the Coast Guard received a number of proposals from companies that wanted their bioremediation products to be tried in Alaska; however, no mechanism existed to enable them to compare competing technologies.[112] One of NETAC'S first charges, therefore, was to recommend criteria by which bioremediation products for cleaning up the Alaska spill could be judged, that is, to develop a protocol for assessing the effectiveness of beach bioremediation products. NETAC convened a panel of experts for this task; from this panel's recommendations, EPA established an official procedure to judge products for possible use in Alaska.

To encourage the submission of products that might qualify for field testing in Alaska in 1990, EPA published an announcement in the *Commerce*

[111] U.S. Environmental Protection Agency, Office of Environmental Engineering and Technology Demonstration, "AlternativeTreatment Technology Information Center" (brochure), June 1990.

[112] J.M. Cogen, "Bioremediation Technology for Spilla," *Waste Business Western*, September 1990, p. 17.

Business Daily.[113] *Thirty-nine* proposals were submitted. The NETAC panel evaluated these and recommended that 11 undergo laboratory testing specified by the protocol. Effectiveness and toxicity tests were conducted in EPA's Cincinnati, Ohio Risk Reduction Engineering Laboratory. EPA, again with NETAC's help, selected two products judged most appropriate for field testing in Alaska.

The process of identifying promising products appears to OTA to be both appropriate and fair and, in general, NETAC is performing a valuable service. Nonetheless, a few of the bioremediation firms that had submitted products contended that the tests specified in the protocol were not appropriate to assess the true effectiveness of these products. As more bioremediation research results become available, it will be possible to refine this frost protocol, if necessary. A NETAC expert panel is currently identifying the kinds of studies and types of tests needed for an open water bioremediation protocol, and EPA's ORD labs will again use the resulting framework protocol to develop a complete experimental design. Although much skepticism remains about the effectiveness of bioremediation at sea, an open water protocol would be useful whether or not any effective products were identified.

Relative to the new Interim Guidelines published by the Oil Spill Response Subcommittee, NETAC is also preparing to assist Regional Response Teams in planning for the possible use of bioremediation technologies. Specifically, NETAC has begun to: 1) systematically compile information on commercial bioremediation products, 2) collect samples for preliminary laboratory evaluation of bioremediation products, 3) define a national bioremediation product evaluation facility to test commercial products, and 4) develop the capability to provide technical assistance to the States and to regional or national response teams.[114]

The Private Sector

The bioremediation industry is a young industry seeking to develop markets for its products and expertise. The industry is composed primarily of companies with fewer than 100 employees, and not many of these companies have been in existence for more than 5 years. Of the companies that have developed bioremediation products, few specialize in products for marine oil spills. If a market for such products were to develop, however, many companies would be interested. More than 50 companies Claiming bioremediation expertise have expressed interest in supplying products or personnel in response to the Persian Gulf oil spill.[115] Only a handful of these products have undergone testing to evaluate their effectiveness on marine spills (see above), and none has yet developed a reputation among experts as an effective response to such spills.

The Applied Biotreatment Association (ABTA) was established in 1989 to promote the interests of the bioremediation industry. The organization has 55 members and consists of about equal numbers of corporate and adjunct associates. Corporate members are biotreatrnent companies, and adjunct members include State biotechnology centers, equipment companies, and university professors. In addition to bioremediation companies, ABTA now includes among its members two large oil companies. ABTA recently produced a briefing paper on the role of bioremediation in oil spills.[116] Several of its members actively participate in EPA's bioremediation Action Committee.

For the most part, the oil industry has taken a wait-and-see attitude toward bioremediation. Few companies are doing research on the bioremediation of marine oil spills, preferring instead to let EPA take the lead. Recently, the Petroleum Environmental Research Forum (PERF), an oil industry group that sponsors research on environmental problems of concern to its members, proposed a mass balance study to evaluate the potential of nutrient enrichment and seeding on open water.[117] This study is expected to begin in mid-1991. Although both the American Petroleum Institute and the new Marine Spill Response Corp. believe that

[113] U.S. Environmental Protection Agency, "EPA Seeks Biological Methods for Potential Application to Cleanup of Alaskan Oil Spill,' Commerce *Business Daily,* Feb. 12, 1990.

[114] National Environmental Technology Applications Corp., "NETAC'S Role in Supporting bioremediation Product Selection for Oil Spills, " Feb. 11, 1991,

[115] K. Devine, Applied Biotreatment Association telephone Communication Jan. 28, 1991.

[116] Applied Biotreatment Association, "The Role of bioremediation of Oil Spills, " fall 1990.

[117] J. Salanitro, Shell Development Co., personal communication Jan. 29, 1991.

bioremediation warrants further research, neither is planning a significant research program of its own.[118] The Exxon Corp. so far is an important exception to the general absence of oil industry activity. Exxon, by virtue of its Alaska and New Jersey experiences, has more familiarity with fertilization techniques than other oil companies and, at least for certain types of environment, has concluded that their use is merited.

REGULATORY ISSUES

Several provisions of the Federal Water Pollution Control Act (the Clean Water Act (CWA)), as recently amended by the Oil Pollution Act of 1990 (OPA), affect or potentially affect the use of bioremediation products for marine oil spills. The CWA specified development of a National Contingency Plan (NCP) for the removal of oil and hazardous substances. The OPA calls for the revision of parts of this plan. It requires EPA to prepare a list of dispersants, other chemicals, and ***other spill mitigating devices and substances*** that may be used to carry out the plan, and to identify the waters in which they may be used and the quantities that can be used safely.[119] Thus, before a bioremediation product could be considered for use in response to an oil spill, it would, at minimum, have to be on this list. Inclusion on the list implies that certain minimum safety standards have been met but does not necessarily imply that the product is either effective or nontoxic for specific applications. In general, additional testing would be required to evaluate further both efficacy and toxicity.

Subpart J of the National Contingency Plan governs the use of biological additives (as well as dispersants and other chemical agents) for marine oil spills. It identifies several options that can be used to obtain authorization for the application of a chemical or biological agent to combat a spill. Section 300.910e provides for preauthorization of the use of regulated agents through an advance planning process. Thus, on-scene coordinators (OSCs) are authorized to use biological additives that have been preapproved by Regional Response Teams. EPA encourages preplanning and believes that the deliberations of Regional Response Teams provide the best forum for considering authorizations.[120]

If a preauthorized plan has not been established or is not applicable to the specific circumstances of a spill, the OSC must obtain the concurrence of the EPA representative to the Regional Response Team, the affected State(s), and impractical, the Department of the Interior and Department of Commerce natural resource trustees, before using biological or other agents. An exception occurs when the OSC determines that quick action is necessary to prevent or substantially reduce a hazard to human life; in that case, the OSC may unilaterally authorize the use of any product, including those not on the NCP product schedule. Continued use of the product once the threat has been mitigated is subject to normal concurrence procedures.[121]

Another statute that may have a bearing on bioremediation technologies is the Toxic Substances Control Act (TSCA). The TSCA is intended to regulate the manufacture of substances that may pose a risk to human health or the environment. The Act applies to *chemical* substances generally, which EPA has interpreted to include microorganisms. Currently, however, manufacturers of microbial products are not required to satisfy Section 5 of TSCA and notify EPA regarding the use of naturally occurring microorganisms, and EPA has no plans to require notification.[122] Genetically engineered organisms, however, would be subject to notification and review. EPA has not had the opportunity to conduct product reviews of genetically engineered organisms, but a few companies are considering their use. EPA expects to publish a draft biotechnology rule in the *Federal Register* by late 1991.

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118 The Marine Spill Response Corp. (MSRC), formerly the Petroleum Industry Response Organization, was established after the *Exxon Valdez oil* spill and is intended to give the private sector an improved capability to respond to major oil spills around the country. MSRC has a sizable research budget.

119 Oil Pollution Act of 1990, section 4201(b). This schedule maybe obtained from EPA's Emergency Response Division. The list currently contains 30 bioremediation products and includes both chemical fertilizers and microbial products.

120 J. Cunningham, U.S. Environmental Protection Agency, presentation to the Treatability Protocol Subcommittee of the bioremediation Action committee, Oct. 15, 1990.

121 Ibid.

122 E. Milewski, U.S. Environmental protection Agency, Office for Pesticides and Toxic Substances, personal communication Mar. *6, 1991.

Appendix A
Glossary

Aerobic bacteria: Any bacteria requiring free oxygen for growth and cell division.

Aliphatic **compound: Any** organic compound of hydrogen and carbon characterized by a linear or branched chain of carbon atoms. Three subgroups of such compounds are alkanes, alkenes, and alkynes.

Anaerobic bacteria: Any bacteria that can grow and divide in the partial or complete absence of oxygen.

Aromatic: Organic cyclic compounds that contain one or more benzene rings. These can be monocyclic, bicyclic, or polycyclic hydrocarbons and their substituted derivatives. In aromatic ring structures, every ring carbon atom possesses one double bond.

Assay: Qualitative or (more usually) quantitative determination of the components of a material or system.

Biodegradation: The natural process whereby bacteria or other microorganisms chemically alter and break down organic molecules.

bioremediation: A treatment technology that uses biological activity to reduce the concentration or toxicity of contaminants: materials are added to contaminated environments to accelerate natural biodegradation.

Catalyst: A substance that alters the rate of a chemical reaction and may be recovered essentially unaltered in form or amount at the end of the reaction.

Cometabolism: The process by which compounds in petroleum may be enzymatically attacked by microorganisms without furnishing carbon for cell growth and division.

Culture: The growth of cells or microorganisms in a controlled artificial environment.

Dispersant: Solvents and agents for reducing surface tension used to remove oil slicks from the water surface.

Emulsification: The process of intimately dispersing one liquid in a second immiscible liquid (e.g., mayonnaise is an example of an emulsified product).

Enzyme: Any of a group of catalytic proteins that are produced by cells and that mediate or promote the chemical processes of life without themselves being altered or destroyed.

Fraction: One of the portions of a chemical mixture separated by chemical or physical means from the remainder.

Gas chromatography: A separation technique involving passage of a gaseous moving phase through a column containing a fixed liquid phase; it is used principally as a quantitative analytical technique for compounds that are volatile or can be converted to volatile forms.

Gravimetric analysis: A technique of quantitative analytical chemistry in which a desired constituent is efficiently recovered and weighed.

Hydrocarbon: One of a very large and diverse group of chemical compounds composed only of carbon and hydrogen; the largest source of hydrocarbons is petroleum crude oil.

Inoculum: A small amount of material (either liquid or solid) containing bacteria removed from a culture in order to start a new culture.

Inorganic: Pertaining to, or composed of, chemical compounds that are not organic, that is, contain no carbon-hydrogen bonds. Examples include chemicals with no carbon and those with carbon in non-hydrogen-linked forms.

Metabolism: The physical and chemical processes by which foodstuffs are synthesized into complex elements, complex substances are transformed into simple ones, and energy is made available for use by an organism; thus all biochemical reactions of a cell or tissue, both synthetic and degradative, are included.

Metabolize: A product of metabolism.

Microorganism: Microscopic organisms including bacteria, yeasts, filamentous fungi, algae, and protozoa.

Mineralization: The biological process of complete breakdown of organic compounds, whereby organic materials are converted to inorganic products (e.g., the conversion of hydrocarbons to carbon dioxide and water).

Oleophilic: Oil seeking (e.g., nutrients that stick to or dissolve in oil).

Organic: Chemical compounds based on carbon that also contain hydrogen, with or without oxygen, nitrogen, and other elements.

Pathogen: An organism that causes disease (e.g., some bacteria or viruses).

Saturated hydrocarbon: A saturated carbon-hydrogen compound with all carbon bonds filled; that is, there are no double or triple bonds, as in olefins or acetylenes.

Soluble: Capable of being dissolved.

Surface-active agent: A compound that reduces the surface tension of liquids, or reduces interracial tension between two liquids or a liquid and a solid; also known as surfactant, wetting agent, or detergent.

Volatile: Readily dissipating by evaporation.

Appendix B
Acknowledgments

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