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.

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.

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).

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 fertil-izer 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

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.

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.)

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.