- 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 oildegrading 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.<sup>°</sup>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).<sup>10</sup> 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.<sup>11</sup> 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,<sup>12</sup> with the loss rate decreasing rapidly over time.<sup>13</sup> 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.<sup>14</sup> 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.<sup>IG</sup>

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 watersoluble 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.<sup>17</sup>

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.

<sup>&</sup>lt;sup>9</sup>For 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 inG.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.

<sup>&</sup>lt;sup>10</sup>National Research Council, op. cit., footnote 1, p. 270.

<sup>&</sup>lt;sup>11</sup>J.R. Payne and G.D. McNabb, Jr., "Weatheringof Petroleum in the Marine Environment,' *Marine Technology Society Journal*, vol. 18, No. 3, 1984, p. 24.

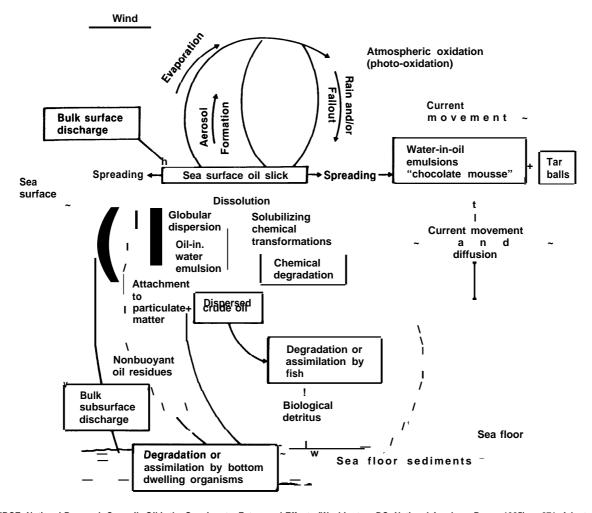
<sup>&</sup>lt;sup>12</sup>National Research Council, op. cit., footnote 1, p. 276.

<sup>&</sup>lt;sup>13</sup>Floodgate, op. cit., footnote 9, p. 362.

<sup>&</sup>lt;sup>14</sup>Payne and McNabb, op. cit., footnote 11, p. 26.

<sup>&</sup>lt;sup>15</sup>Floodgate, op. cit., footnote 9, p. 362.

<sup>&</sup>lt;sup>17</sup>National 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.<sup>18</sup> 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, <sup>19</sup> 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.<sup>20</sup>

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

<sup>&</sup>lt;sup>18</sup>Payne and McNabb, op. cit., footnote 11, p. 30.

<sup>19</sup> National Research Council, Marine Board, Using Oil Dispersants on the Sea (Washington, DC: National Academy Press, 1989).

<sup>&</sup>lt;sup>20</sup>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.<sup>21</sup>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.<sup>22</sup> 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.<sup>25</sup> 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.<sup>26</sup> 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.<sup>28</sup> 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

<sup>&</sup>lt;sup>21</sup>Mielke, Op. cit., footnote 16, P. 12.

<sup>&</sup>lt;sup>22</sup>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.

<sup>&</sup>lt;sup>23</sup>R.M. Atlas, "Biodegradation of Hydrocarbons in the Environment," in *Environmental Biotechnology*, G.S. Omenn (cd.), (New York, NY: Plenum Press, 1988), p. 214.

<sup>24</sup> National Research Council, Op. Cit., footnote 1, p. 290.

<sup>&</sup>lt;sup>25</sup>J.J. Cooney, "Microbial Ecology and Hydrocarbon Degradation" paper presented at the Alaska Story Symposium, Cincinnati, OH, Sept. 17-18, 1990., p. 2.

<sup>&</sup>lt;sup>26</sup>National Research Council, op. cit., footnote 1, p. 17.

<sup>27</sup>Atlas, op. cit., footnote 23, p. 212.

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

others.<sup>29</sup> 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.<sup>30</sup> 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.<sup>31</sup> 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.<sup>32</sup>

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.<sup>33</sup>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.<sup>34</sup> High-molecular-weight aromatics comprise 2 to 10 percent of light oils and up to 35 percent of heavy oils.<sup>35</sup>

The asphaltic fraction contains compounds that either are not biodegradable or are degraded very slowly.<sup>36</sup>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.<sup>37</sup>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.<sup>38</sup>

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.<sup>39</sup> 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-molecularweight alkanes, 5 percent light aromatics, 20 percent high-molecular-weight aromatics, 10 percent asphaltenes, and 10 percent resins.<sup>40</sup>

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.<sup>41</sup> 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

<ul> <li><sup>29</sup>Atlas, op. cit., footnote 23, p. 212.</li> <li><sup>30</sup>Cooney, Op. cit., footnote <sup>25</sup>.</li> </ul>
<sup>31</sup> Atlas, op. cit., footnote 23, p. 212.
<sup>32</sup> M. Fingas, Environment Canada, personal communication, Oct. 5, 1990.
<sup>33</sup> Ibid.
<sup>34</sup> National Research Council, op. cit., footnote 1, p. 293. <sup>35</sup> Fingas, Op. cit., footnote 32.
<sup>36</sup> Cooney, op. cit., footnote 25, p. 3.
<sup>37</sup> Cooney, op. cit., footnote 25, p. 3; and National Research Council, op. cit., footnote <sup>1</sup> , P. 295. <sup>38</sup> Fingas, Op. cit., footnote <sup>32</sup> .
<sup>39</sup> Leahy and Colwell, op. cit., footnote 28, p. 305.
<sup>40</sup> 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.<sup>42</sup>

# 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.<sup>43</sup>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.<sup>44</sup>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.<sup>45</sup>

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.<sup>®</sup> Lack of an appropriate enzyme either prevents attack or is a barrier to complete hydrocarbon degradation.

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

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

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.<sup>47</sup>)

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-

<sup>44</sup>Lee and Levy, op. cit., footnote 22, pp. 217-243. <sup>45</sup>Cooney, op. cit., footnote 25, p.1.

<sup>46</sup>Applied Biotreatment Association, "The Role of Biotreatment of Oil Spills," rev. 2, August 1990, p. 4.

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

<sup>&</sup>lt;sup>42</sup>Cooney, op. cit., footnote 25, P-3.

<sup>&</sup>lt;sup>43</sup>Floodgate, OD. cit., footnote 9, p. 372.

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.<sup>48</sup> 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.<sup>49</sup>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.<sup>50</sup>

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.<sup>52</sup> Although petroleum is rich in the carbon required by microorganisms, it is deficient in the mineral nutrients necessary to support microbial growth.<sup>53</sup> Marine and other ecosystems are often deficient in these substances because non-oildegrading microorganisms (including phytoplankton) consume them in competition with the oildegrading 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.<sup>54</sup>

52Lee and Levy, Op. cit., footnote 22, p. 223.

<sup>&</sup>lt;sup>48</sup>Leahy and Colwell, op. cit., footnote 28, p. 307.

<sup>&</sup>lt;sup>49</sup>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.

<sup>&</sup>lt;sup>50</sup>Lee and Levy, op. cit., footnote 22, p. 224.

<sup>51</sup> Cooney, op. cit., footnote 25, p. 4.

<sup>53</sup>Atlas, Op. cit., footnote 23, p. 216.

<sup>54</sup>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.<sup>55</sup> 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 dramaticall, 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.<sup>56</sup> At low temperature, the rate of hydrocarbon metabolism by microorganisms decreases.<sup>57</sup> 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.<sup>58</sup>

### **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.<sup>59</sup>

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.<sup>60</sup>

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. 1—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.<sup>61</sup>

<sup>55</sup>Floodgate, Op. cit., footnote 9, p. 381.

<sup>56</sup>Ibid.

<sup>&</sup>lt;sup>57</sup>Leahy and Colwell, op. cit., footnote 28, p. 307.

<sup>58</sup>Floodgate, OD. cit., footnote9, p. 381-

<sup>&</sup>lt;sup>59</sup>J.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.

<sup>&</sup>lt;sup>60</sup>Lee and Levy, op. cit., footnote 22, p. 225.

<sup>61</sup>Leahy and Colwell, op. cit., footnote 28, p. 308.

#### 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.

# 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).<sup>62</sup> 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.<sup>63</sup> 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.<sup>64</sup> Fourth, bioremediation of oil spills is accomplished on-site, and offers a simpler and more thorough solution to polluted areas. In contrast, hot water

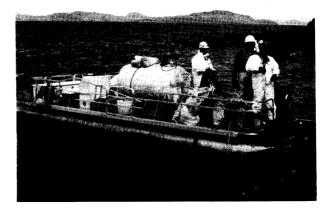


Photo credit: Exxon Corp.

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

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 *shortterm* 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

<sup>&</sup>lt;sup>62</sup>These attributes are discussed in the Applied Biotreatment Association, "BriefmgPaperonthe Role of bioremediation of Oil Spills," rev. 2, August 1990.

<sup>63&</sup>lt;sub>M.S.</sub> 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.

<sup>64</sup>J. Glaser, Environmental Protection Agency, Risk Reduction Engineering Laboratory, personal communication, Feb. 20, 1991.