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Status of Sea-Borne Bioremediation Technologies

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I. INTRODUCTION

Bioremediation is a relatively new approach for cleaning up oil pollution in the marine environment without undesirable side effects. Drawing upon a variety of disciplines, such as microbiology, ecology, toxicology, biotechnology, and chemistry,[1] bioremediation aims at accelerating biodegradation of oil compounds. Current bioremediation methods applied *in situ* fall under three major categories, to be discussed in Section II. This chapter summarizes information available on the biodegradation of targeted compounds, outlines historical, chemical, and biological aspects of marine bioremediation, and discusses examples of field bioremediation attempts.

Oil spills have catastrophic effects to most marine ecosystems. Light penetration decreases to just a few centimeters underneath the oil slick. Photosynthesis is thus inhibited throughout most of the euphotic zone and phytoplankton-based food webs are severely impaired. The oil slick blocks gas exchanges between the water and the atmosphere, reducing dissolved oxygen to levels insufficient to support aerobic life forms. Several oil compounds are toxic to aquatic life forms, while air inlets, and hence respiration of higher invertebrates, can be obstructed entirely by oil. Using detergents to clean the fur or feathers of water dwelling animals can lead to drowning by counteracting the waterproofing effect of oily secretions. Large-scale application of detergents as oil dispersants can affect an even greater variety of life forms by increasing biological membrane permeability to toxic compounds. The decomposition of dead organisms releases large amounts of nutrients, which in turn cause eutrophication in areas adjacent to the spill.[2] Petroleum compounds with a specific gravity only slightly higher than that of the water form the oil-in-water emulsion that is often washed up on beaches as tar balls.[3]

Of the 3 billion tons of petroleum produced annually worldwide, 1.7–8.8 million tons of hydrocarbons enter the marine environment.[4] In the period 1975–1985, average annual petroleum input to the sea due to a number of tanker accidents was 240,000–400,000 tons or approximately 0.02–0.03 percent of the 1.4 billion tons transported annually by sea (Table 1).

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Table 1 Accidental Oil Spills from Tankers, 1975–1985

Year	Amount transported (10⁶ tons)	Total amount spilled (tons)	No. of oil spills	Name of ship	Flag	Location	Cause	Amount spilled (tons)
1975	188,042		45	Showa Maru	Japan	Strait of Malacca	Grounding	4,000
				Br. Ambassador	UK	Pacific coast, Japan		45,000
				Jakob Maersk	Denmark	off Portugal, Atlantic		84,000
				Corinthos/E.M.	USA/Liberia	Delaware coast, Atlantic		40,000
				Queeny				
1976	204,235		29	Spartan Lady	Liberia	USA		25,000
				Epic Colocotroni	Greece	St. Dominique		57,000
				Saint Peter	Liberia	Columbia		33,000
				Urquiola	Spain	Spain		101,000
				Cretan Star	Cyprus	Indian Ocean		28,600
				Boehlen	GDR	off Brittany	Sinking	11,000
1977	213,000		49	Argo Merchant	Liberia	Massachusetts		28,000
				Irenes Challenge	Liberia	Pacific		34,000
				Borag	Liberia	East China coast		4,000
				Hawaiian Patriot	Liberia	Honolulu, Pacific		99,000
				Caribean Sea	Panama	Nicaragua		30,000
				Venoil/Venpet	Liberia	40 km off South Africa	Collision	26,000
				Grand Zenith	Panama	Massachusetts		29,000
1978	260,488		35	Amoco Cadiz	Liberia	off Brittany, France	Grounding	228,000
				Cabo Tamar	Chile	Talcahuano, Chile	Grounding	60,000
				Christos Bitas	Greece	Pembrokeshire, UK		5,000
				Andros Patria	Greece	off Cape Villano, Spain	Explosion, fire	47,000
1979	723,533		65	Betelgeuse	France	Bantry Bay, Ireland	Explosion	27,000
				Antonio Gramsci	USSR	300 km south of Finland	Grounding	6,000
				Messianiki Frontis	Liberia	south of Crete, Mediterranean	Grounding	6,000
				Kurdistan	UK	Cabot Strait, Canada	Ramming iceberg	8,000
				Gino	Liberia	off Brittany, France	Collision	42,000
1980	135,635		32	Aviles	Liberia	Arabian Sea, India	Explosion	25,000
				Atlantic Express/	Greece/Liberia	Tobago Island, Caribbean Sea	Collision	276,000
				Aegean Captain				
				Ioannis Angelicoussis	Greece	Cabinda, Angola	Fire, explosion	30,000
				Burmah Agate	Liberia	outside Galveston Bay, Texas	Collision, fire	40,000
				Independenta	Romania	outside Instanbul harbor, Turkey	Collision, fire	94,600
				Fortune	Liberia	90 km northeast of Singapore	Collision	10,000
				Astoria	Greece	Emden, GFR	Ramming	40,000
				Princess Anne Maraz	Greece	off SW tip of Cuba	Grounding	6,000
				Irene Serenade	Greece	Navarino Bay, Greece	Explosion, fire	102,000
				Tanio	Madagascar	60 km off Brittany coast, France	Heavy sea	13,500
1981	45,285		33	Juan A. Lavaglia	Uruguay	Algeria, Mediterranean	Grounding	40,000
				Jose Marti	USSR	Sweden, Baltic Sea	Grounding	6,000
				Globe Assimi	USSR	USSR, Baltic Sea		16,000
				Cavo Cambanos	Greece	France		18,000
1982	1,716		9	Fukutoku Maru, #8	Japan	Tachibana Bay, Japan	Collision	85
				Sivona	Sweden	SW coast of Sweden	Collision	088
				Arkas	Liberia	Mississippi River, near Montz	Collision fog	530
1983	387,773		17	Castellode de Belvar	Spain	110 km NE of Cape Town, S. Africa	Fire/fracture	50,000
				Sivand	Iran	Humber River, UK	Ramming	7,000
				Feoso Ambassador	China	South China Sea, off Hong Kong	Grounding	4,000
				Monemvasia	Greece	South China Sea, off Singapore	Grounding	1,000
				PNCO Basilan	Philippines	South China Sea, off Agoo	Grounding	17,062
1984	24,184		15	Assimi	Greece	90 km NE of Oman	Fire, sinking	51,431
				Pericles G.C.	Greece		Fire, capsize	46,631
1985	400,000		1363	Nova	Liberia	Arabian Gulf	Collision	71,120
				Neptunia	Liberia	off Bushire, Iran	Missile attack	60,000
				Grand Eagle	USA	Delaware River, USA	Grounding	1,436

When inputs from petroleum production, transportation, and refining operations are added, the annual amount of hydrocarbons entering the seas increases considerably (Table 2). The impact of tanker accidents is most pronounced in closed waters, such as the Mediterranean Sea, through which the majority of the oil is transported from the oil-exporting to the oil-importing countries.[4]

The number of annual oil spills in U.S. sea routes does not exceed 5 percent of the total number of oil spills worldwide, while the amount of oil potentially threatening U.S. waterways never has exceeded one-tenth of the global annual average. The 12 most catastrophic accidents over a 17-year period (1973–1990) in the Gulf of Mexico/Galveston Bay are shown in Table 3. With the exception of the 1979 blowout of the Ixtoc oil well, which spilled approximately 350,000 tons of crude oil, burned over a nine-month period, and accounted for over 90 percent of the all-time total oil input in the Gulf of Mexico/Galveston Bay, an average of 5000 tons of petroleum entered the Gulf of Mexico/Galveston Bay annually over this period.

Sea-borne bioremediation first appeared in 1967, when contractors attempted to improve the quality of an oil–wastewater mixture in the refuse tanks of the *Queen Mary*.[7] The technology was further advanced after the 1968 *Amoco Cadiz* oil spill off the coast of Brittany with the commercial development of Inipol EAP-22, a liquid oleophilic fertilizer.[6] In the 1970s, biological treatment was regarded unsuitable for the remediation of oil spills at sea because of the complexity and unpredictability of biological responses to environmental factors. Decision makers relied almost exclusively on physicochemical processes such as containment of the oil slick, recovery by skimming and/or pumping, dispersant application, or in situ burning. These methods, however, were often limited by high operational costs, equipment unavailability near the oil spill site, adverse weather conditions, etc. Improved understanding of microbial processes, reduced costs, as well as growing public demand for environmentally sound technologies, made bioremediation an attractive alternative and/or supplemental oil spill cleanup method.

After the 1968 *Amoco Cadiz* oil spill, most of the knowhow that was used in the cleanup

Table 2 Estimated World Input of Petroleum Hydrocarbons (10^6 tons/yr) to the Sea

Source	Total
Transportation	1.45
Tanker operations	0.70
Tanker accidents	0.40
Bilge and fuel oils	0.30
Dry docking	0.03
Nontanker accidents	0.02
Fixed installations	0.37
Coastal refineries	0.10
Offshore production	0.05
Marine terminals	0.02
Other sources	1.38
Municipal wastes	0.70
Industrial waste	0.20
Urban runoff	0.12
River runoff	0.04
Atmospheric fallout	0.30
Ocean dumping	0.02
Natural inputs	0.25
Total	3.25

Source: Ref. 4.

Table 3 The 12 Most Catastrophic Spills in the Gulf of Mexico/Galveston Bay, 1973–1990

Year	Accident	Type	Site	Cause	Amount spilled ($\times 10^3$ L)
1973	Mayo Lykes	Tanker	Houston Ship Channel	Collision	1,520
1979	IXTOC I	Oil field	Southern Gulf of Mexico	Blowout	668,556
1979	Burmah Agate	Tanker	Galveston Bay	Collision	40,553
1980	Georgia reg.	Tanker	Pilottown, Louisiana	Anchor chain	4,927
1981	Olympic Glory	Tanker	Galveston Bay	Collision	3,790
1982	Arkas	Tanker	Monte, Louisiana	Collision	5,590
1984	Alvenus	Tanker	Cameron, Louisiana	Hull cracks	10,612
1988	AMOCO	Pipeline	30 mi SE of Galveston	Burst	2,210
1988	Nord Pacific	Tanker	Corpus Christi Channel	Collision	2,445
1989	Rachel B	Tank barge	Houston Sip Channel	Collision	948
1990	Mega Borg	Tanker	57 mi SE of Galveston	Explosion	17,434
1990	Apex	Tank barge	Galveston Bay	Collision	2,710

Source: Ref. 6.

of the *Exxon Valdez* and *Mega Borg* accidents, came from soil and ground water bioremediation efforts because

Terrestrial systems provide better control of experimental parameters. Parameters such as temperature, evaporation, wind, currents, or wave action are crucial to the outcome of bioremediation, but virtually uncontrollable in the marine environment.

Not all marine oil spills can be subject to study and experimentation. Many spills remain undetected or are discovered late. Even when promptly detected, they may remain inaccessible for prolonged periods of time, during which they are subject to chemical weathering or other physicochemical processes whose impact cannot be assessed.

The frequency of marine oil spills is relatively low compared with that of terrestrial spills. The consequences of the latter can be more immediate to humans. For example, terrestrial oil spills often result in contamination of groundwater used as drinking water supply, and their cleanup is an issue of higher priority to humans. .

The term *bioremediation* includes any technology, methodology, or other human intervention intended to accelerate naturally occurring biodegradation in contaminated environments.[7] *Biodegradation* is defined as the set of metabolic pathways by which organisms modify and/or decompose natural or anthropogenic organic molecules into other compounds, such as fatty acids or carbon dioxide.[7] In the present chapter, the term *sea-borne* refers to any locale within or in contact with sea water, such as beaches, marine sediments, mangroves, estuaries, mud flats, or the water–air interface.

II. SEA-BORNE BIOREMEDIATION TECHNOLOGIES

Currently, sea-borne bioremediation technologies fall under three general categories: (1) fertilizer addition, (2) seeding with naturally occurring biodegraders, and (3) introduction of genetically engineered microorganisms.

A. Fertilizer Addition

Fertilizers can be utilized to overcome nutrient limitation of microbial communities. In field applications this method has provided effective cleanup, relatively free of unwanted complications such as inducement of eutrophication and toxicity.

The bioremediation of the March 1989 *Exxon Valdez* oil spill at Prince William Sound, Alaska is the best available example of bioremediation using nutrient enrichment. The steps taken in the in situ cleanup effort are briefly discussed here.[7]

Step 1: Treatability. In this step preliminary work was carried out to determine if nutrient application could accelerate degradation of oil by enhancing growth of beach microbial communities. Oil biodegradation was assessed in several test field sites by monitoring changes in oil composition, vertical and horizontal movement of added nutrients, and bacterial abundance.

Step 2: Treatment. On 1 August 1989, after the treatability studies determined that fertilizer use would be both effective and safe, fertilizers were applied to approximately 100 miles of polluted beaches in the Sound. Three fertilizer types were used:

1. A slow release, water-soluble fertilizer in various forms and sizes
2. A water-soluble fertilizer applied with a sprinkler system
3. A liquid oleophilic fertilizer, applied with sprayers, previously developed to keep the nutrients and the oil in contact

Step 3: Short-Term Evaluation. Studies were conducted by Exxon Corp. and the U.S. EPA to evaluate short-term effectiveness of Exxon's treatment and application program.

Step 4: Long-Term Evaluation. EPA, Exxon Corp., and the Alaska Department of Environmental Conservation combined efforts to monitor the long-term recovery of the treated beaches.

Step 5: Further Research. Additional studies were undertaken to examine the potential of adding microorganisms to the polluted beaches for accelerated biodegradation.

After the first fertilizer application in March 1990, surface sediment microbial numbers were not different between untreated and treated plots. The number of hydrocarbon degraders in the subsurface sediments of treated plots, however, as well as hexadecane and phenanthrene mineralization rates were significantly higher than in untreated plots. A second fertilizer application, later in the summer, resulted in increased surface and subsurface biodegrader counts compared with untreated sediments in control sites. The higher number of hydrocarbon degraders, along with the elevated mineralization potential, indicated that hydrocarbon biodegradation was enhanced, although these measurements alone are not sufficient to determine in situ rates of crude oil biodegradation.[9]

Another bioremediation attempt by Exxon at Prall's Kill, New Jersey, used a novel technique of fertilizer application. Two trenches parallel to the water line were excavated at different distances from the shore and were filled with fertilizer. The incoming tide dissolved the fertilizer, which was subsequently washed down by the ebb, achieving a more even distribution throughout the beach. Three months after treatment initiation, oil degradation in the treated zone was significantly higher than in untreated areas.

B. Seeding with Naturally Occurring Biodegraders

There are two variants of this technology. In the first, oil-degrading microorganisms are isolated from the oil spill site by screening. The selected microbes are subsequently cultivated and reinoculated into the site. In the second, the site is inoculated with biodegraders from other natural environments previously exposed to oil pollution. In either case, nutrients are supplied as needed to maximize the effects of biodegradation.

On 8 June 1989, the Norwegian tanker *Mega Borg* exploded in open waters approximately 60 miles off Galveston Island, spilling 17.5 million liters of Angolan light crude into the Gulf of Mexico, generating a 35-mile-long slick that drifted for days. Treatment consisted of spraying

the oil slick with 50 kg of a commercially available microbial medium (Alpha BioSea Process) containing naturally occurring microorganisms (10^9 bacteria/g dry weight of preparation), diluted in 208 L of sea water and supplemented with inorganic nutrients (nitrogen and phosphorus). Within hours of treatment application, the oil had emulsified in the experimental area and the most hazardous hydrocarbons had been degraded. Next morning, the slick had almost completely disappeared. Analysis of water samples taken before and after the treatment, as well as from untreated areas, revealed that no sample (pretreatment, posttreatment, or control) contained any acute toxicity.[6] Therefore, it was uncertain whether the disappearance of the slick was the result of bioremediation and/or the skimming operations performed overnight in the area. The disappearance of oil from control areas suggests that the effect of bioremediation in this case was minimal, probably because of the short time involved.

In July 1989, the Greek tanker *Shinoussa*, laden with highly explosive jet fuel, collided with three Apex tank barges loaded with crude oil and dumped 2.7 million liters of its contents into the Texas bay and estuary system. In addition to mechanical methods of oil removal (skimming and booming), seeding with microorganisms was successfully used to limit the extent of the oil slick.[6]

C. Introduction of Genetically Engineered Microorganisms

This method focuses on incorporating genes regulating widely differing metabolic pathways in a single microorganism species. The resulting *genetic cassettes* can be transferred to appropriate hosts and, in theory, provide more efficient biodegradation during emergencies.

The engineering of *superbugs*, organisms that can consume a wide variety of organic pollutants, was challenged by the finding that aromatic and aliphatic hydrocarbons are broken down by entirely different microorganisms.[10] Several genes coding for enzymes with broad substrate specificity for aromatic compounds have been cloned, however, and the construction of degradative operons has also been reported.[11]

III. PETROLEUM-DEGRADING ORGANISMS

The excessive amounts of hydrocarbons found in marine oil spills can stimulate heterotrophic microbial activity.[12] Various heterotrophic and saprotrophic microorganisms, such as bacteria and fungi, are capable of metabolizing petroleum hydrocarbons, an attribute that makes these microorganisms valuable bioremediation tools.

Bacteria are by far the most important category of petroleum-degrading organisms. They can derive energy through organic matter metabolism by transferring electrons from an electron donor (in the case of oil, hydrocarbons) to an electron acceptor (oxygen, chlorine, nitrates, sulfur). Because of their generally short generation times and lack of protective mechanisms and structures to mutagenic factors (e.g., UV radiation, chemicals), bacteria and fungi undergo mutations more frequently than other organisms. Some mutations result in advantageous genetic changes, which become fixed throughout the population, thus enhancing its biodegradation efficiency. Such mutations are currently the research objective of many developing bioremediation technologies.

Fungi, together with bacteria, are responsible for the majority of hydrocarbon biodegradation in the soil.[13] The role of fungi in marine bioremediation is less critical, however. Various fungi isolated from temperate and tropical habitats are capable of utilizing petroleum hydrocarbons as the sole carbon source.[14] Beach-adapted species of *Corollospora*, *Dendryphiella*, *Lulworthia*, and *Varicosporina*, as well as a few other fungi isolated from submerged wood

and salt marsh vegetation, grew using alkanes and alkenes as the sole carbon source, and mineralized $n[1-^{14}C]$hexadecane.[15]

The importance of other microorganisms in bioremediation is minor compared with that of bacteria and fungi. Protozoan predation affects biodegradation of toxic and hazardous materials in soil, surface, groundwater, and wastewater.[16] Wave action or emulsification may generate oil droplets of appropriate size to be ingested by zooplankton, which seem unaffected by the presence of oil. As much as 20 percent of the oil can be sedimented to the bottom by fecal pellets of relatively high specific density.[17] Algae, although capable of developing resistance to organic pollutants, do not play an important role in oil biodegradation, mainly because of their autotrophic nutrition mode. Some algae, however, possess other mechanisms for removing toxic organic compounds from the marine environment. The diatoms *Ditylum brightwellii* and *Thalassiosira nordenskoldii* isolated from polychlorinated biphenyls (PCBs) contaminated coastal waters responded quite differently to increasing concentrations of these lipophilic compounds; the former developed resistance by sequestering PCBs in a cell compartment isolated from physiological activity, while the latter reduced its intracellular PCBs accumulation by decreasing the lipid:carbon ratio within the cell.[18] These mechanisms are mentioned here as examples of the wide variety of biological responses to pollutants and the potential for exploiting microorganisms in novel detoxification technologies.

IV. BIODEGRADATION OF OIL COMPOUNDS

Crude oil is a complex mixture of over 1000 organic compounds widely differing in molecular weight, structure, and physicochemical properties. Low molecular weight alkanes (ethane, propane, and butane), cyclohexanes (naphthenes), aromatics (with one or more benzene or heterocyclic rings per molecule), asphaltenes, and resins make up the majority of crude oil.[12] Depending on origin, petroleum may contain variable trace amounts of PCBs, sulfur, and heavy metals such as nickel, vanadium, and lead, making the composition of each oil unique.

The chemical and abiotic aspects of petroleum biodegradation have been extensively studied and are well documented.[19,22] The results of selected studies on biodegradation of several petroleum compounds are summarized in Tables 4 and 5. Hydrocarbons containing up to 44 C atoms are susceptible to various degrees of biodegradation with compounds of 10–24 C atoms being most degradable.[7] Readily biodegradable hydrocarbon categories include short-chain aliphatic hydrocarbons such as alcohols and ketones; aromatic compounds such as benzene, toluene, xylene, creosote, and phenolics; and lighter halogenated aromatics such as chlorinated benzenes and pentachlorophenol.[8] Heavier hydrocarbons such as asphalts, waxes, and tars are not easily broken down.[8] The higher molecular weight chlorinated hydrocarbons, including PCBs and pesticides, are also resistant to biodegradation.[4] The resistance of these compounds to biodegradation is due to the high stability of the carbon–chlorine bond and the thermodynamic energetics involved.[52] Dehalogenation and metabolism are two biochemical processes important for the detoxification and biodegradation of petroleum compounds, and they are briefly described here.

Dehalogenation, the removal of halogens from hydrocarbons, can be stimulated by (1) introduction of electron donor and acceptor combinations, (2) nutrient additions, (3) engineered microorganisms, and (4) use of enzyme systems catalyzing reductive dehalogenation.[53] Rates and pathways of carbon tetrachloride (CT) reductive dehalogenation by *Escherichia coli* depend on the electron acceptor present; aerobic or nitrate-respiring conditions result in inhibition of [^{14}C]CT metabolism, low oxygen levels result in $^{14}CO_2$ production and cell-bound ^{14}C, whereas increasingly reductive conditions result in decreasing amounts of $^{14}CO_2$ and increasing amounts

Table 4 Biodegradation of Selected Organic Compounds

Group	Family	Compound	Biodegradation	Reference
Aliphatic	Alkanes	Hexadecane Heptadecane Nonadecane	A strain of the bacterium *Pseudomonas aeruginosa* isolated from an oil spillage degraded hexadecane, heptadecane, octadecane and nonadecane by 47%, 58%, 73%, and 60%, respectively. Carbon dioxide evolving from the respective substrates was 9%, 12%, 27%, and 25%, while biosurfactant production by the bacterium reduced surface tension of hexadecane to approximately one-half the surface tension of water.	Ref. 23
	Chlorinated		Up to 50% of total organic and low molecular weight halides were removed in an aerated lagoon treating kraft mill waste water. The remaining organics were adsorbed on settling biomass and transported to the bottom of the lagoon where they were anaerobically degraded and dehalogenated.	Ref. 24
Polycyclic aromatic hydrocarbons (PAHs)			Preexposure of marine sediment slurries to relatively high concentration of a particular polycyclic aromatic hydrocarbon resulted in the enhanced degradation rate of that PAH as well as some other PAHs. This enhanced biodegradation rate has been attributed to the selective proliferation of microbial populations capable of degrading a broad range of PAHs or having common pathways for PAH degradation.	Ref. 25
		Anthracene	A mixed bacterial community isolated from a marine hydrocarbon-polluted site was not capable of degrading anthracene, but proved capable of utilizing anthraquinone, a by-product of anthracene photooxidation.	Ref. 26
		Fluoranthene	A *Mycobacterium* sp. isolated from oil-contaminated marine sediments was capable of mineralizing up to 78% fluoranthene after 5 days of incubation with relatively little intermediate metabolites. Following a 6–12 hour delay period the *Mycobacterium* degraded over 95% of the fluoranthene.	Ref. 27
			A stable community of seven bacteria isolated from a coal tar contaminated soil was capable of degrading fluoranthene as the sole carbon source. Subsequent incubation of the above community with a mixture of 16 PAHs representative of creosote contamination and fluoranthene resulted within three days in degradation of 13 PAHs below the level of detection (10 ng/L).	Ref. 28

Table 4 Continued

Group	Family	Compound	Biodegradation	Reference
		Naphthalene	New strains of *Pseudomonas* isolated from marine sediments of a heavily polluted area with naphthalene did not show 1,2-dioxygenase activity but catechol 2,3-dioxygenase was responsible for breaking down the aromatic ring.	Ref. 29
			Naphthalene mineralization half-life in laboratory microcosms was 2.4 weeks for sediments chronically exposed to oil pollution compared with 4.4 and 3.2 weeks in sediments from unpolluted and agricultural-chemicals receiving sites, respectively. Although total heterotrophic bacterial populations at the site exposed to agricultural chemicals showed a 30-fold increase compared with the unpolluted site, hydrocarbon-utilizing micro-bial populations at the sediments of the petrochemically and agriculturally polluted sites were 5–12 and 2–3 times greater than the unpolluted site, respectively.	Ref. 30
		Phenanthrene	Degradation of high phenanthrene concentrations by estuarine water and sediment microbial populations seems to be a two-stage process: first, phenanthrene is degraded with the produc-tion of biomass and intermediate aromatic hydrocarbons, and second consumption of these hydrocarbons and a secondary increase in biomass. Various nonionic surfactants solubilized phenanthrene and enhanced its utilization by a *Mycobacterium* sp. isolated from the sediments, but degradation did not corre-late with degree of solubility indicating interference of sur-factant with the physiological processes of the *Mycobacterium*.	Ref. 31
			Rates and amounts of phenanthrene and naphthalene mineralized by microbial communities from sediments of intertidal marine basins were strongly correlated with oxygen availability, pre-exposure time and compound concentration, whereas nitrogen and glucose amendments had no effect on mineralization.	Ref. 32

Group	Family	Compound	Biodegradation	Reference
		Pesticides	Microbial communities in estuarine sediments may degrade a variety of pesticides. The degradation of nine of the 14 pesti-cides proceeded significantly faster in the presence of sediment slurry than in the absence of it, whereas only two pesticides degraded faster in the absence of sediment than in the presence of it and only two degraded by abiotic processes.	Ref. 33
		Methyl parathion	Sediments from estuaries in Florida and Mississippi did not differ significantly in degradation rates of methyl parathion, pointing to the fact that spatial variations are of limited importance in determining the environmental fate of this insecticide.	Ref. 34
		Phenols, chlor-ophenols	Several types of chlorophenols were mineralized by estuarine sediment inocula under sulfate-reducing conditions with the production of sulfide. Because no methane was formed, the sulfate must have been the electron acceptor. Mineralization proceeded much faster in subsequent refeeding episodes after initial stimulation of the microbial community with chlorophe-nols. However, chloroanilines could be reductively de-halogenated by microorganisms in methanogenic but not sulfate-reducing conditions.	Refs. 35, 36
Phenyls	Chlorinated biphenyls (PCBs)		In contrast to DDT and some other insecticides, polychlorinated biphenyls have never been introduced into the environment intentionally but accidentally by leakage, incineration, and dumping of wastes at sea. Large amounts of PCBs in landfills and on the bottom of the sea form a pool of release for years to come, while lipophilic unmetabolized PCB residues in the bios-phere are not likely to disappear for many decades. Increasing chlorination increases the lipophilicity and extent of cytochrome P-450 binding, while it decreases the metabolism and excretion of these molecules. Persistence of these compounds depends on the degree of chlorination and position of chlorine atoms.	Refs. 37, 38
		Asphaltenes	Even the recalcitrant asphaltenes have been reported to undergo biodegradation by a mixed marine bacterial population with saturated hydrocarbons as a co-substrate, but this has been attributed to the character of the particular oil rather than the action of bacteria.	Ref. 39

Table 5 Cometabolism of Organic Oil Pollutants

Substrate	Inducer	Enzyme	Organism	Reference
Dichloromethane	Methane	sMMO	Methylosinus trichosporium OB3b	Refs. 41–44
	Ammonia	AMO	Nitrosomonas europaea	
Trichloromethane	Methane	sMMO	Methylosinus trichosporium OB 3b	Refs. 41–44
	Ammonia	AMO	Nitrosomonas europaea	
Monochloroethylene	Methane	sMMO	Methylosinus trichosporium OB 3b	Refs. 41–45
	Ammonia	AMO	Nitrosomonas europaea	
1,1-Dichloroethylene	Methane	sMMO	Methylosinus trichosporium OB 3b	Refs. 41,42,45
	Propane	PMO	Mycobacterium spp.	
	Ammonia	AMO	Nitrosomonas europaea	
cis-1,2-Dichloroethylene	Methane	sMMO	Methylosinus trichosporium OB 3b	Refs. 41,42,45
	Propane	PMO	Mycobacterium spp.	
trans-1,2-Dichloroethylene	Methane	sMMO	Methylosinus trichosporium OB 3b	Refs. 41,42,45
	Propane	PMO	Mycobacterium spp.	
1,1-Dichloroethane	Methane	sMMO	Methylosinus trichosporium OB 3b	Refs. 41,42
1,2-Dichloroethane	Methane	sMMO	Methylosinus trichosporium OB 3b	Refs. 41,42
1,1,2-Trichloroethane	Ammonia	AMO	Nitrosomonas europaea	Refs. 43,44,46
	Camphor	cytochrome P450	Pseudomonas putida PpG-786	
1,1,1-Trichloroethane	Ammonia	AMO	Nitrosomonas europaea	Refs. 43,44
Trichloroethylene	Methane	sMMO	Methylosinus trichosporium OB 3b	Refs. 41–44,47,48
	Ammonia	AMO	Nitrosomonas europaea	
	Toluene	MO	Pseudomonas cepacia G4	
	Phenol	TO	Pseudomonas putida F1/Escherichia coli	
	Propane	PMO	Mycobacterium spp.	
2,4-dichloro-phenoxy-acetic acid			Alcaligenes eutrophus JMP134	Refs. 45,49,50
1,2-Dichloropropane	Methane	sMMO	Methylosinus trichosporium OB 3b	Refs. 41,42,51
1,2,3-Trichloropropane	Ammonia	AMO	Nitrosolobus multiformis	
	Ammonia	AMO	Nitrosomonas europaea	Refs. 43,44
1,2-Dibromo-3-chloropropane	Ammonia	AMO	Mycobacterium spp.	Ref. 44

Source: Ref. 40.

of chloroform and membrane-bound ^{14}C.[54] Anoxic dehalogenation of halogenated phenols by microbial communities of marine sediments gave the following results: (1) dehalogenation proceeded in the order para- to meta- to ortho-forms; (2) dehalogenation rates increased between 6–30°C and decreased at higher temperatures; (3) bromophenols degraded faster than chlorophenols; and (4) sediment previously exposed to a pulp and paper mill effluent showed increased biodegradation rates.[55]

During *aerobic metabolism*, organic matter breakdown proceeds at relatively fast rates. Aeration is often applied in bioremediation attempts to boost the rate of aerobic metabolism. Air pumped through unsaturated soils, with simultaneous addition of fertilizers and moisture increased aerobic degradation rates.[56] A venturi system providing aeration and mixing of microbial communities with a water-in-oil emulsion from marine mud contaminated with oil from the *Christos Bitas* spillage resulted in 95 percent degradation of the solvent extractable oil after 83 days.[57] Oxygen availability can also be increased through hydrogen peroxide application.[58,60] Hydrogen peroxide applied to gasoline-contaminated soil stimulated biodegradation of benzene, toluene, ethylbenzene, and o-xylene (BTEX), with toluene being mineralized most and xylene least.[61]

Anaerobic metabolism is generally considered slow compared with aerobic processes. It often produces intermediate products that are more toxic than the reactants and require further treatment. Some low molecular weight aromatic hydrocarbons, however, can be degraded anaerobically.[62,63]

In *cometabolism*, microorganisms transform compounds without growing on the compound alone or without deriving energy, carbon, or any other nutrient from the transformation.[54] In methanotrophs and nitrifiers, the degradation of chlorinated aliphatics by mono- and dioxygenases is induced by entirely different compounds (Table 5).

V. FACTORS AFFECTING BIODEGRADATION

Oil hydrocarbons provide an abundant source of raw materials for bacterial metabolism. Microbial growth, however, may be limited by a variety of other factors, such as temperature, nutrient and oxygen availability, degree of compound chlorination, degree of branching, number of aromatic rings, availability of electron donors and acceptors, and size, concentration, and toxicity of organic compounds.

Temperature affects many enzymatic processes, and it influences biodegradation in rather complicated ways. Biodegradation practically ceases near the freezing point, while it proceeds rapidly in warm and calm seas. Biodegradation rates increase as temperature increases up to 27°C. Further increases, however, may bring the process to a halt. Increased bacterial activity may quickly deplete meager dissolved oxygen levels and switch biodegradation to the much slower anaerobic pathways. At lower temperatures, the oil slick becomes more viscous, reducing the area available for contact with microorganisms. Slow metabolic rates combined with reduced contact area result in drastically decreased biodegradation rates, despite the higher dissolved oxygen concentrations in colder waters.

Oxygen availability determines the type of metabolism (aerobic or anaerobic; see Section IV), thus affecting greatly biodegradation rates. In the absence of oxygen, nitrate can be utilized as the electron acceptor in microbial respiration with contaminant hydrocarbons as the carbon source. Nitrate is more soluble and less costly than oxygen, and less toxic than hydrogen peroxide. Several recalcitrant compounds (BTEX) are effectively degraded in the presence of nitrate.[64] Enhanced aerobic phenol biodegradation by microbial communities previously exposed to phenol was observed when nitrate instead of oxygen was added as the final electron acceptor.[65]

Biodegradation rates decrease with increasing degree of chlorination, branching, and number of aromatic rings. Several heavier compounds and metals are directly toxic to the microorganisms, thereby inhibiting biodegradation for prolonged periods.

VI. TARGETED SYSTEMS

The value of bioremediation in open waters relative to other remediation methods is usually limited. Physicochemical processes such as wave action, dispersion, evaporation, and photooxidation are the main processes affecting the size and impact of the oil spill at sea. The most important of these processes during the first 48 hours of an oil spill is usually evaporation of the volatile fraction, accounting for as much as 30–60 percent weight loss depending on the origin and composition of the crude oil.[7] Formation of oil droplets in rough seas results in increased surface area for adsorption of microorganisms and sufficient oxygenation for microbial activity. Biodegradation may be severely limited, however, because of the difficulties in keeping the oil slick contained and the microorganisms in contact with the oil droplets. In calm open seas, containment of the spill is easier and skimmers can be used to recover the oil. In such cases, implementation of oil recovery technologies can yield significant financial savings. When recovery is not possible, and if conditions permit, in situ burning can be used alternatively. Controlled bioremediation, however, can be applied effectively in more confined systems, such as beaches, estuaries, sediments, and mangroves.

A. Beaches

The most significant beach bioremediation effort to date has been that of *Exxon Valdez*, described in Section II.A.

B. Estuaries

Biodegradation of m-cresol and nitrilotriacetic acid (NTA) by aerobic heterotrophic bacteria was greatest in an estuary and lowest in a marine site.[66] Similar results have been reported for chlorobenzene and 1,2,4-trichlorobenzene with biodegradation rates being relatively uniform at the marine site throughout the year.[67] Biodegradation was important in determining the fate of m-cresol and NTA in the warmer part of the year and at low concentrations, whereas at higher concentrations and at the marine site other processes such as adaptation, dilution, and mixing of pollutants were more significant.[66]

Biodegradation of NTA in a Canadian estuary with a history of preexposure to NTA was the result of indigenous bacterial populations and was not affected by dissolved oxygen and salinity fluctuations.[68]

C. Sediments

Aerobic bacteria isolated from marine sediments were capable of metabolizing various forms of phthalate in the presence of Na^+ ions by forming hydroxylated compounds, probably through the utilization of phthalate hydroxylases.[69] Degradation of p-chlorophenol (p-CP) in estuarine sediments may not be an entirely biotic process, but it is enhanced by bacterial activity.[70] Previous exposure of water and sediment bacteria to p-CP resulted in higher p-CP degradation rates, while aerobic conditions seem to favor p-CP biodegradation in the sediments.[70] Microbial population growth and denitrification in sediments has been simulated by imbedding the marine denitrifier *Pseudomonas nautica 617* in a carageenan gel.[71] The gel populations had both a shorter generation time and a higher nitrate reduction activity compared with free-living bacteria

(1.7 hours and 7 μmol NO_3/h/mg protein vs. 2.4 hours and 4.6 μmol NO_3/h/mg protein, respectively).

D. Mangroves

Biodegradation of crude oil hydrocarbons was very slow in an experimentally polluted peaty mangrove substrate, despite the periodical artificial oxygenation applied. As a result of slow mineralization of organic matter, biodegradation was limited by the absence of nutrients. Addition of an oleophilic fertilizer stimulated aerobic microbial activity and increased alkane biodegradation.[72]

VII. DISCUSSION

Bioremediation is a relatively new approach for restoring damage caused by oil spills in the marine environment leaving, according to accumulated evidence, minimal or no undesirable side effects. It is expected to play an increasingly important role in future management of oceanic systems because of its low cost, ease of application, and environmental soundness. Large corporations, such as Exxon and General Electric, have been conducting bioremediation in an attempt to repair the impact of their activities to the environment. Under growing public pressure, more corporations are likely to develop bioremediation technologies not only for internal company use, but also for marketing these technologies to other parties. For example, Lockheed Co. is in the process of testing a new bioremediation technology that accelerates biodegradation rates by improving oil contact with bacteria.[73] A powdered clay provides increased surface for adsorption of microorganisms and simultaneously coagulates oil, thereby preventing its sinking. The advantage of this method is that large amounts of clay can be delivered per plane or shipload. Preliminary tests have shown that in the presence of fertilizer about 75 percent of the oil is degraded after 36 days.

Although many technological advances arise from field bioremediation attempts, the empirical knowledge acquired by each attempt is not necessarily applicable to other cases.[7] For example, fertilizer application was effective in the *Exxon Valdez* oil spill, but did not yield spectacular results in the spill of the Spanish tanker *Aragon*. Speculation attributed performance differences to the different oil type and origin, fertilizer concentration, geomorphological features of the sites, prevailing climatic conditions, and degree of initial microbial activity. Because of each environment's uniqueness of biological, geomorphological, and chemical characteristics and the limited degree of existing field bioremediation experience,[74] treatability studies are required before any in situ bioremediation effort is considered.[58] However, the following steps have been proposed as a generalized bioremediation action scheme:[7]

1. *Compliance analysis*. This involves understanding what regulations apply to the particular problem at hand and what actions need to be taken.

2. *Thorough site and waste investigation and assessment*.[75] The source, nature, distribution, and concentration of contaminants in the site must be examined, as well as the type and volume of material to be treated.

3. *Feasibility and engineering studies (= treatability)*. The decision is made here as to which of the available remediation technologies can be most effectively applied to remediate the particular contaminants.

4. *Remediation*. Selected cleanup strategy is implemented.

5. *Monitoring*. The effectiveness of the applied treatment and the restoration progress of the polluted system are monitored.

Proponents of the inoculation-with-natural-biodegraders method claim that specific assemblages of microorganisms can be prepared against any kind of petroleum or pollutant combination. These special assemblages can be maintained or stored frozen for prolonged periods and deployed whenever circumstances arise. The main disadvantage of the method is the long acclimation time of microbial assemblages previously unexposed to the environmental conditions of the site. Although microbial response time during the *Mega Borg* crisis was approximately 30 min,[6] the same period may be significantly prolonged in other cases. Moral and legal issues may further complicate the use of this technology. Local conservation laws often prohibit the introduction of living specimens from another state or part of the country. The prevailing belief is that, given enough time and nutrients, naturally occurring microbial communities will break down most organic compounds released by the spill. Recent advances, however, continuously reveal new biochemical pathways and strains capable of degrading compounds previously believed recalcitrant. For example, a new strain of *Mycobacterium* capable of metabolizing the relatively recalcitrant 3-, 4-, and 5-ring polycyclic aromatic hydrocarbons has recently been discovered.[76-79]

Biotreatment by introducing genetically engineered microorganisms has never been implemented at full scale. Its use is still regarded as highly controversial,[80] and its popularity is decreasing among scientists and public. Initial enthusiasm about the concept of the *genetic cassettes* has been replaced by skepticism about the safety and effectiveness of such an approach.[7] On technical grounds, there is no immediately available technology that can start from the targeted oil compound and, working in a reverse direction, assemble the genes responsible for degrading that compound. Pressure against the use of this technology also comes from public concern about the possible introduction of "laboratory monsters" in the environment. Public reluctance to accept this technology is likely to postpone its application in the near future.

REFERENCES

1. Grady, C. P. L., Biodegradation of toxic organics: Status and potential. *J. Env. Eng. ASCE*, *116*, 805–828 (1990).

2. Shales, S., Thake, B. A., Frankland, B., Khan, D. H., Hutchinson, J. D., and Mason, C. F., Biological and ecological effects of oils, in *The Fate and Effects of Oil in Freshwater* (J. Green and M. Trett, eds.), Applied Science Publishers, London, 1989, pp. 81–171.

3. Baker, J. H., and Griffiths, R. P., Effects of oil on bacterial activity in marine and freshwater sediments, in *Current Perspectives in Microbial Ecology: Proceedings of the Third International Symposium on Microbial Ecology* (M. J. Klug and C. A. Reddy, eds.), American Society for Microbiology, Washington, D.C., 1984, pp. 546–551.

4. Clark, R. B., *Marine Pollution*, 2nd ed., Oxford Science Publications, Oxford, 1989.

5. United Nations Environmental Program, *Environmental Data Report*, 2nd ed., Blackwell, Oxford, 1989.

6. Texas General Land Office, *Bioremediation: Nature's Cleanup Tool*, Austin, Texas, 1991.

7. U.S. Congress, *Bioremediation for Marine Oil Spills*, OTA-BP-O-70, Office of Technology Assessment, U.S. Government Printing Office, Washington, D.C., 1991.

8. Thayer, A. M., Bioremediation: Innovative technology for cleaning up hazardous waste. *Chem. Eng. News*, August 26, 1991, pp. 23–44.

9. Lindstrom, J. E., Prince, R. C., Clark, J. C., Grossman, M. J., Yeager, T. R., Braddock, J. F., and Brown, E. J., Microbial populations and hydrocarbon biodegradation potentials in fertilized shoreline sediments affected by the T/V Exxon Valdez oil spill. *Appl. Env. Microbiol.*, *57*, 2514–2522 (1991).

10. Foght, J. M., Fedorak, P. M., Westlake, D. W., Mineralization of [14C]phenanthrene in crude oil: specificity among bacterial isolates. *Can. J. Microbiol.*, *56*, 169–175 (1990).

11. Chaudry, G. R., and Chapalamadugu, S., Biodegradation of halogenated organic-compounds, *Microbiol. Rev.*, 55, 59–79 (1991).

12. Mason, C. F., *Biology of Freshwater Pollution*, 2nd ed., Longman Scientific & Technical Group, New York, 1991.

13. Atlas, R. M., and Atlas, M. C., Biodegradation of oil and bioremediation of oil spills, *Curr. Opinion in Biotech.*, 2, 440–443 (1991).

14. Kirk, P. W., Dyer, B. J., and Noe, J., Hydrocarbon utilization by higher marine fungi from diverse habitats and localities, *Mycologia*, 83, 227–230 (1991).

15. Kirk, P. W., and Gordon, A. S., Hydrocarbon degradation by filamentous marine higher fungi, *Mycologia*, 80, 776–782 (1988).

16. Alexander, M., *Biodegradation of Chemicals at Trace Concentrations*, ADA-212 409/7, National Technical Information Service, Springfield, Virginia, 1989.

17. Conover, R. J., Some relations between bunker C oil in Chedabucto Bay following the wreck of the tanker Arro, *J. Fish. Res. B. Can.*, 28, 1327–1330 (1971).

18. Cohen, M. K., West, A. S., Cosper, E. M., and Wurster, C. F., Mechanisms of resistance to polychlorinated-biphenyls (PCB) in 2 species of marine diatoms, *J. Mar. Biol. Assoc.*, 71, 247–263 (1991).

19. Colwell, R. R. and Walker, J. D., Ecological aspects of microbial degradation of petroleum in the marine environment, *CRC Crit. Rev. Microbiol.*, 423–445 (1977).

20. Atlas, R. M., Stimulated petroleum degradation, *CRC Crit. Rev. in Microbiol.*, 371–386 (1977).

21. Neilson, A. H., The biodegradation of halogenated organic compounds, *J. Appl. Bacteriol.*, 69, 445–470 (1990).

22. Leahy, J. G., and Colwell, R. R., Microbial degradation of hydrocarbons in the environment, *Microbiol. Rev.*, 54, 305–315 (1990).

23. Shafeeq, M., Kokub, D., Khalid, Z. M., Khan, A. M., and Malik, K. A., Degradation of different hydrocarbons and production of biosurfactant by *Pseudomonas aeruginosa* isolated from coastal waters, *Mircen J. Appl. Microbiol. Biotech.*, 5, 505–510 (1989).

24. Amy, G. L., Bryant, C. W., Alleman, B. C., and Barkley, W. A., Biosorption of organic halide in a kraft mill generated lagoon, *J. Water Poll. Control Fed.*, 60, 1445–1453 (1988).

25. Bauer, J. E., and Capone, D. G., Effects of co-occurring aromatic hydrocarbons on degradation of individual polycyclic aromatic hydrocarbons in marine sediment slurries, *Appl. Env. Microbiol.*, 54, 1649–1655 (1988).

26. Rontani, J. F., Rambeloarisa, E., Bertrand, J. C., and Giusti, G., Favourable interaction between photooxidation and bacterial degradation of anthracene in sea water, *Chemosphere*, 14, 11–12 (1985).

27. Kelley, I., and Cerniglia, C. E., The metabolism of fluoranthene by a species of *Mycobacterium*, *J. Industrial Microbiol.*, 7, 19–26 (1991).

28. Mueller, J. G., Chapman, P. J., and Pritchard, P. H., Action of a fluoranthene-utilizing bacterial community on polycyclic aromatic hydrocarbon components, *Appl. Env. Microbiol.*, 55, 3085–3090 (1989).

29. Garcia-Valdes, E., Cozar, E., Rotger, R., Lalucat, J., and Ursing, J., New naphthalene-degrading marine *Pseudomonas* strains, *Appl. Env. Microbiol.*, 54, 2478–2485 (1988).

30. Heitkamp, M. A., Freeman, J. P., and Cerniglia, C. E., Naphthalene biodegradation in environmental microcosms: Estimates of degradation rates and characterization of metabolites, *Appl. Env. Microbiol.*, 53, 129–136 (1987).

31. Guerin, W. F., and Jones, G. E., Mineralization of phenanthrene by a *Mycobacterium* sp. *Appl. Env. Microbiol.*, 54, 937–944 (1988).

32. Bauer, J. E., and Capone, D. G., Degradation and mineralization of the polycyclic aromatic hydrocarbons anthracene and naphthalene in intertidal marine sediments, *Appl. Env. Microbiol.*, 50, 81–90 (1985).

33. Walker, W. W., Cripe, C. R., Pritchard, P. H., and Bourquin, A. W., Biological and abiotic degradation of xenobiotic compounds in in vitro estuarine water and sediment/water systems, *Chemosphere*, 17, 2255–2270 (1988).

34. Pritchard, P. H., O'Neil, E. J., Spain, C. M., and Ahearn, D. G., Physical and biological parameters that determine the fate of p-chlorophenol in laboratory test systems, *Appl. Env. Microbiol.*, 53, 1833–1838 (1987).

35. Haggblom, M. M., and Young, L. Y., Chlorophenol degradation coupled with sulfate reduction, *Appl. Env. Microbiol.*, 56, 3255–3260 (1990).

36. Kuhn, E. P., and Sulfita, J. M., Sequential reductive dehalogenation of chloroanilines by microorganisms from a methanogenic aquifer, *Env. Sci. Tech.*, 23, 848–852, (1989).

37. Bickel, M. H., Polychlorinated persistent compounds, *Experientia*, 38, 879–882 (1982).

38. Bickel, M. H., and Muhlebach, S., Persistent and unmetabolizable lipophilic compounds: From pharmacokinetics to ecotoxicology, in *Chlorinated Dioxins and Related Compounds*, 1982, pp. 303–306.

39. Rontani, J. F., Bosser-Joulak, F., Rambelarisa, E., Bertrand, J. C., Giusti, G., and Faure, R., Analytical study of Asthart crude oil asphaltenes biodegradation, *Chemosphere*, 14, 1413–1422 (1985).

40. Young, L. Y., and Haggblom, M. M., Biodegradation of toxic and environmental pollutants, *Curr. Opinion Biotechnol.*, 429–435 (1991).

41. Tsien, H. C., Brusseau, G. A., Hanson, R. S., and Wackett, L. P., Biodegradation of trichloroethylene by *Methylosinus trichosporium* OB3b, *Appl. Env. Microbiol.*, 55, 3155–3161 (1989).

42. Oldenhuis, R., Vink, R. L. J. M., Janssen, D. B., and Witholt, B., Degradation of chlorinated aliphatic hydrocarbons by *Methylosinus trichosporium* OB3b expressing soluble methane monooxygenase, *Appl. Env. Microbiol.*, 55, 2819–2826 (1989).

43. Vannelli, T., Logan, M., Arciero, D. M., and Hooper, A. B., Degradation of halogenated aliphatic compounds by the ammonia-oxidizing bacterium *Nitrosomonas europea*, *Appl. Env. Microbiol.*, 56, 1169–1171 (1990).

44. Rasche, M. E., Hyman, M. R., and Arp, D. J., Biodegradation of halogenated hydrocarbon fumigants by nitrifying bacteria, *Appl. Env. Microbiol.*, 56, 2568–2571 (1990).

45. Wackett, L. P., Brusseau, G. A., Householder, S. R., and Hanson, R. S., Survey of microbial oxygenases: Trichloroethylene degradation by propane-oxidizing bacteria, *Appl. Env. Microbiol.*, 55, 2960–2964 (1989).

46. Castro, C. E., and Belser, N. O., Biodehalogenation: oxidative and reductive metabolism of 1,1,2-trichloroethane by *Pseudomonas putida*—biogeneration of vinyl chloride, *Env. Toxicol. Chem.*, 9, 707–714 (1990).

47. Nelson, M. J. K., Montgomery, S. O., Mahaffey, W. R., and Pritchard, P. H., Biodegradation of trichloroethylene and involvement of an aromatic biodegradation pathway, *Appl. Env. Microbiol.*, 53, 949–954 (1987).

48. Folsom, B. R., Chapman, P. J., and Pritchard, P. H., Phenol and trichloroethylene degradation by *Pseudomonas ceparia* G4: Kinetics and interactions between substrates, *Appl. Env. Microbiol.*, 56, 1279–1285 (1990).

49. Zylstra, G. J., Wackett, P., and Gibson, D. T., Trichloroethylene degradation by *Escherichia coli* containing the cloned *Pseudomonas putida* F1 toluene dioxygenase genes, *Appl. Env. Microbiol.*, 55, 3162–3166 (1989).

50. Harker, A. R., and Kim, Y. K. Trichloroethylene degradation by two independent aromatic-degrading pathways in *Alcaligenes europhus* JMP134, *Appl. Env. Microbiol.*, 56, 1179–1181 (1990).

51. Rasche, M. E., Hicks, R., Hyman, R. M., and Arp, D. J., Oxidation of monohalogenated ethanes and n-chlorinated alkanes by whole cells of *Nitrosomonas europea*, *J. Bacteriol.*, 172, 5368–5373 (1990).

52. Atlas, R. M. and Bartha, R., *Microbial Ecology: Fundamentals and Applications*, Addison-Wesley, Reading, Massachussetts, 1981.

53. Sims, J. L., Suflita, J. M., and Russel, H. H., *Reductive Dehalogenation: A Subsurface Bioremediation Process*, PB91-144873, National Technical Information Service, Springfield, Virginia, 1990.

54. Criddle, C. S., deWitt, J. T., and McCarty, P. L., Reductive dehalogenation of carbon tetrachloride by *Escherichia coli* K-12, *Appl. Env. Microbiol.*, 56, 3247–3254 (1990).

55. Abrahamsson, K., and Klick, S., Degradation of halogenated phenols in anoxic natural marine sediments, *Mar. Poll. Bull.*, 22, 227–233 (1991).

56. Hinchee, R. E., and Arthur, M., Bench scale studies of the soil aeration process for bioremediation of petroleum hydrocarbons, *Appl. Biochem. Biotech.*, 28–29, 901–906 (1991).

57. Berwick, P. G., Pilot trials of the microbial degradation of Christos-Bitas water in oil emulsion (chocolate mousse) and BP Llandarcy gas oil using venturi. *Biotech. Bioeng.*, 27, 94–108 (1985).

58. Raymond, R. L., Lee, M. D., and Ward, C. H., Assessment of potential for in-situ bioremediation, in *DECHEMA Biotechnology Conferences*, vol. 3A (D. Behrens and A. J. Driesel, eds.), DECHEMA, Frankfurt Am Main, Germany, 1990, pp. 41–44.

59. Wilson, J. T., Kampbell, D. H., Armstrong, J., Raymond, R. L., Thomas, J. M., Fiorenza, S., Rifai, H. S., Bedient, P. B., and Ward, C. H., Quantitative field evaluation of bioremediation of a fuel spill using hydrogen peroxide, in *DECHEMA Biotechnology Conferences*, vol. 3A (D. Behrens and A. J. Driesel, eds.), DECHEMA, Frankfurt Am Main, Germany, 1990, pp. 45–48.

60. Huling, S. G., Bledsoe, B. E., and White, M. V., *Enhanced Bioremediation Utilizing Hydrogen Peroxide as a Supplemental Source of Oxygen: A Laboratory and Field Study*, PB90-183435, National Technical Information Service, Springfield, Virginia, 1990.

61. Thomas, J. M., Gordy, V. R., Fiorenza, S., and Ward, C. H., Biodegradation of BTEX in subsurface materials contaminated with gasoline: Granger, Indiana. *Water Sci. Tech.*, 22, 53–62 (1990).

62. Grbic-Galic, D., and Vogel, T. M., Transformation of toluene and benzene by mixed methanogenic cultures, *Appl. Env. Microbiol.*, 53, 254–260 (1987).

63. Bertrand, J. C., Caumette, P., Mille, G., Gilewicz, M., and Denis, M., Anaerobic degradation of hydrocarbons, *Sci. Program*, 73, 333–350 (1989).

64. Hutchins, S. R., Downs, W. C., Smith, G. B., Wilson, J. T., and Hendrix, D. J., *Nitrate for Biorestoration of an Aquifer Contaminated with Jet Fuel*, PB91-16285, National Technical Information Service, Springfield, Virginia, 1991.

65. Britton, L. N., *Aerobic Denitrification as an Innovative Method for In Situ Biological Remediation of Contaminated Subsurface Sites*, ADA-208 81218, National Technical Information Service, Springfield, Virginia, 1989.

66. Palumbo, A. V., Placender, F. K., and Pearl, H. W., Biodegradation of NTA and m-cresol in coastal environments, *Env. Toxicol. Chem.*, 7, 573–585 (1988).

67. Bartholomew, G. W., and Placender, F. K., Influence of spatial and temporal variations on organic pollutant biodegradation rates in an estuarine environment, *Appl. Env. Microbiol.*, 45, 103–109 (1983).

68. Larson, R. J., and Ventullo, R. M., Kinetics of biodegradation on nitrilotriacetic acid (NTA) in an estuarine environment, *Ecotox. Env. Safety*, 12, 166–179 (1986).

69. Taylor, B. F., and Amador, J. A., Metabolism of pyridine compounds by phthalate-degrading bacteria, *Appl. Env. Microbiol.*, 54, 2342–2344 (1988).

70. Pritchard, P. H., Cripe, C. R., Walker, W. W., Spain, J. C., and Bourquin, A. W., Biotic and abiotic degradation rates of methyl parathion in freshwater and estuarine water and sediment samples, *Chemosphere*, 16, 1509–1520 (1987).

71. Bonin, P., Barbotin, J. N., Dhulster, P., and Bertrand, J. C., Nitrate reduction in simulated microniches by a denitrifying bacterium, *Can. J. Microbiol.*, 33, 276–279 (1987).

72. Scherer, P., and Mille, G., Biodegradation of crude oil in an experimentally polluted peaty mangrove soil, *Mar. Pollut. Bull.*, 20, 430–432 (1989).

73. *Environmental Science and Technology*, A new method for applying bioremediating microbes to ocean oil slicks, *Env. Sci. Tech.*, 26, 421 (1992).

74. Gee, J. R., Feasibility of bioremediation at hazardous waste sites, in *Proceedings of Annual Madison Waste Conference*, 11, 235–256 (1988).

75. Sims, J. L., Sims, R. C., and Matthews, J. E., Approach to bioremediation of contaminated soil, *Haz. Waste Mat.*, 7, 117–149 (1990).

76. Heitkamp, M. A., and Cerniglia, C. E., Polycyclic aromatic hydrocarbon degradation by a *Mycobacterium* sp. in microcosms containing sediment and water from a pristine ecosystem, *Appl. Env. Microbiol.*, 55, 1968–1973 (1989).

77. Keck, J., Sims, R. C., Coover, M., Park, K., and Symons, B., Evidence for cooxidation of polynuclear aromatic hydrocarbons in soil, *Water Res.*, 23, 1467–1476 (1989).

78. Wang, S., and Bartha, R., Effect of bioremediation of polycyclic aromatic hydrocarbon residues in soil, *Env. Sci. Tech.*, 24, 1086–1089 (1990).

79. Cerniglia, C. E., Campbell, W. I., Freeman, J. P., and Evans, F. E., Identification of a novel metabolite in phenanthrene metabolism by the fungus *Cunninghamella elegans*, *Appl. Env. Microbiol.*, 55, 2275–2279 (1989).

80. Zitrides, T. G., *Bioremediation Comes of Age. Pollution Engineering*, Cahners Publishing Co., 1990.