Appl Microbiol Biotechnol (1997) 48: 677–686

MINI-REVIEW

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Petroleum hydrocarbon bioremediation: sampling and analytical techniques, in situ treatments and commercial microorganisms currently used

Received: 10 June 1997 / Received revision: 14 August 1997 / Accepted: 25 August 1997

Abstract The sampling and analytical methods, along with available microorganisms, used for in situ hydrocarbon bioremediation are reviewed. Each treatment method is briefly described and its advantages and limitations pertaining to potential applications are evaluated. Bioremediation provides cost-effective, contaminant- and substrate-specific treatments equally successful in reducing the concentrations of single compounds or mixtures of biodegradable materials. In situ treatments rarely yield undesirable byproducts, but precautions and preliminary baseline tests are always recommended. Sampling methods should adhere to good laboratory and field practices and usually do not require highly trained personnel. Analytical methods vary in sensitivity, cost, duration of sample analysis and personnel training required. Voucher specimens of bacterial strains used in bioremediation exist in various repositories (e.g. ATCC, DSM, etc.) or are commercially available, and are usually covered by patent rights. Each one of these strains may yield spectacular results in vitro for specific target compounds. However, the overall success of such strains in treating a wide range of contaminants in situ remains limited. The reintroduction of indigenous microorganisms isolated from the contaminated site after culturing seems to be a highly effective bioremediation method, especially when microorganism growth is supplemented by oxygen and fertilizers.

Introduction

Bioremediation is a rapidly developing field of environmental restoration, utilizing natural microbial activity to reduce the concentration and/or toxicity of various chemical substances such as petroleum products, aliphatic and aromatic hydrocarbons (including polyaromatic hydrocarbons and polychlorinated biphenyls), industrial solvents (phenols, benzene, acetone etc.), battery liquids, pesticides, and metals (arsenic, chromium, selenium, etc.).

Biodegradation is a natural process carried out by soil and aquatic microorganisms – mostly bacteria and fungi. Certain bacterial strains have a demonstrated ability to break down or transform the chemical substances present in petroleum products. Oil-spill bioremediation methods aim at providing favorable conditions of oxygen, temperature and nutrients to maximize biological hydrocarbon breakdown. Such methods have been applied successfully in restoring polluted seashores, airports, military operations, power plants, etc.

This paper is a short overview of the commonest petroleum hydrocarbon bioremediation methods currently used. Emphasis has been placed on sampling and analytical methodologies (Table 1), microorganisms (Table 2), and treatment enhancement by supplying nutrients, oxygen, etc. (Table 3).

Sampling precautions

Hydrocarbon samples are subject to chemical, biological, and physical changes as soon as they are collected. Sample handling, preservation, and storage techniques should minimize changes in sample composition by retarding chemical and/or biological activity. Contamination of sediment samples during collection and handling should be avoided. A detailed procedure for sampling equipment operation and sample handling and storage should be clearly stated in the sampling plan. This may be accomplished by using standard operating procedures [U.S. Environmental Protection Agency (EPA) 1995]. For example, samples designated for hydrocarbon analysis should not come into contact with plastic surfaces.

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Sample contamination from vessel emissions and sampling apparatus must be avoided. Core samples are recommended for the determination of contamination at different depths. Water samples should be collected with either a non-contaminating pump or a discrete water sampler. When sampling with a pump, using a peristaltic pump or one with a magnetically coupled impeller can minimize contamination. Water samplers should be made of stainless steel or acrylic plastic, while seals should be Teflon-coated whenever possible. Prior to sample collection, water sampling devices should be rinsed by a solvent that does not react with the sampler material. For extended storage, freezing in solid CO_2 or a freezer is recommended. For shorter periods of storage, refrigeration at 4 °C is sufficient.

Hydrocarbon analysis

Sediment samples are dried overnight at 60 °C. Residual oil is extracted in chloroform for 8 h using a Soxhlet apparatus. Total oil content is estimated by weighing the dry residue after solvent evaporation. Subsequently, the extract is sonicated in 60 ml hexane (5 min, 40 kHz). The hexane-insoluble fraction contains asphaltenes, which are obtained by filtration on solvent-washed, preweighed Whatman GF/A glass-microfibre filters. The asphaltenes retained on the filters are, in turn, dried and weighed. The hexane-soluble fraction (maltenes) is separated by solid/liquid chromatography on a 15-cm length × 1-cm diameter silica gel column (100–200 mesh), activated overnight at 110 °C. The saturated petroleum hydrocarbons, aromatics and resins are successively eluted with 60 ml hexane, 60 ml 60:40 (v/v) mixture of hexane/dichloromethane and 60 ml methanol. After evaporation of the solvents, each fraction is weighed and the relative percentage of the four classes (saturates, aromatics, resins, and asphaltenes) can be calculated. The saturated and aromatic fractions are analyzed by computerized capillary gas chromatography using a gas chromatograph with a direct-injection port, equipped with a flame ionization detector and a capillary column (50 m × 0.32 mm, film thickness 0.25 μm); He at 0.8 bar is the carrier gas. The column temperature is programmed from 100 °C to 320 °C at 3 °C/min for the saturated fraction, and from 100 °C to 300 °C at 3 °C/min for the aromatics (Oudot 1994).

A variant of the aforementioned analytical procedure uses a column packed with silica gel, where the hexane-soluble fraction is partitioned into saturated, aromatic and polar fractions (Mille et al. 1991). Each fraction is weighed using a microbalance. Saturated hydrocarbons are analyzed by capillary gas chromatography. The apparatus used is equipped with a capillary column (30 m; 0.32 mm inner diameter), an on-column injector and a flame ionization detector. Temperature increase is programmed from 70 °C to 300 °C at 5 °C/min, with helium as the carrier gas at 0.5 bar. Aromatic hydrocarbons are analyzed by UV synchronous luminescence spectroscopy on a spectrophotometer. Polar compounds are analyzed by UV fluorescence and Fourier-transform infrared spectroscopy (FTIR) on a spectrophotometer, whereas asphaltenes are analyzed by FTIR.

The fraction containing the saturated and aromatic hydrocarbons is separated by capillary gas chromatography using standards. *n*-Alkanes are identified by comparing their retention times with those of standard *n*-alkanes ranging from *n*-C_{11} to *n*-C_{31}. Concentrations of individual hydrocarbons are determined by calculating the surface area underneath the sample peak of the respective constituent, identified from its R_F. The content in each *n*-alkane ($n < 31$) is determined as the ratio of the area under the corresponding sample peak to the area under the peak of internal standards.

The aromatic fraction is characterized by gas chromatography/mass spectrometry (GC/MS) (Venosa et al. 1991). Some investigators (Huesemann 1995) utilize a comprehensive petroleum characterization procedure involving group-type-separation analyses, boiling-point distributions, and hydrocarbon typing by field desorption mass spectroscopy to determine initial and final concentrations of specified hydrocarbon classes.

Currently available information on analytical techniques employed for quantitative determination of petroleum hydrocarbons (Table 1) distinguishes methods for (a) the measurement and identification of contaminants, and (b) the measurement of microbial activity. The former are very important because they provide baseline information including the quantity and type of contaminants, distribution patterns, treatability, etc. The latter monitor biological activity and provide an assessment of biodegradation. When information from both methods is combined, the effectiveness of bioremediation can be evaluated.

Petroleum hydrocarbon contamination usually exists as a complex mixture of hydrocarbons. Despite the huge potential of microorganisms to degrade organic compounds under favorable conditions, no single species of microorganism can degrade all the components of a given oil (Office of Technology Assessment, 1991), and no oil-degrading "superbug" has been engineered. Currently, several organisms are known, each capable of degrading usually one or, at best, a few petroleum components at a time. Therefore, effective bioremediation of petroleum contamination requires a mixture of populations consisting of different genera each capable of metabolizing the respective compounds. Table 2 summarizes information on some commercially available bacterial and fungal strains used for petroleum hydrocarbon bioremediation. The bioremediation capacity of bacteria has been investigated more extensively because they are (1) easier to culture, (2) more amenable to molecular biology techniques, (3) capable of metabolizing chlorinated organics, (4) capable of mineralizing these chemicals and using them as carbon energy sources (Bouwer and Zehnder 1993). Although capable of metabolizing some aromatic contaminants, fungi require a primary growth substrate, such as glucose or cellulose to

Table 1 Analytical Techniques

Name	Short description	Parameter measured	Applicable to	Advantages	Constraints	Reference
Soil respirometry	The rate of crude oil biodegradation in a soil matrix is assessed by measuring the rate of carbon dioxide production and oxygen consumption in a soil respirometer	Microbial activity	Soils with low organic matter content	Appears to provide accurate estimates of contaminant biodegradation kinetics	The addition of petroleum hydrocarbons to high-organic-carbon soils may result in enhanced biodegradation of soil organic matter that cannot by accounted for in a control experiment	Huesemann and Moore 1994
Electrolytic respirometry	An electrolytic respirometry apparatus is comprised of a temperature-controlled water bath containing measuring units, a recorder for digital indication and direct plotting of the oxygen uptake velocity curves, and a cooling unitfor the conditioning and continuous recirculation of water bath volume. Each measuring unit is comprised of a reaction vessel with a carbon dioxide absorber mounted inside, an oxygen generator connected by tubing, and pressure indicators connected electronically to the unit. Several measuring units can be monitored simultaneously and data stored in a computer. The measuring units are interconnected by tubing, forming an air-sealed system. Depletion of oxygen by microbial activity within each measuring unit creates a drop in pressure. Oxygen production is triggered by the reduced pressure and sufficient oxygen is produced to counterbalance oxygen loss. The electrical current used to generate the oxygen is measured by the digital recorder, and the data are converted directly into mg/l oxygen uptake. The CO_2 produced by microbial activity is absorbed by soda lime. The nitrogen/oxygen ratio in the gas phase above the sample is maintained constant throughout the experiment, and there is no depletion of oxygen	Microbial activity	Soils with low organic matter content	Atmospheric pressure fluctuations do not adversely affect the results	Same as above	Venosa et al. 1991
Gas chromatography	This technique separates a complex mixture of organic materials into its components. The sample extract is injected into a heated chamber, in which the mixture is concentrated at the head of a separating column. The mixture is then carried through the column by an inert gas (mobile phase). As the column is heated, the analytes pass through absorbent materials (stationary phase). From the chromatogram and the digital information contained in the quantification report, analytes contained in the sample can be accurately identified and quantified	Contaminant identification and determination	Complex extracts of oil, containing hundreds, even thousands, of components	Satisfactory separation of complex mixtures, high sensitivity, accurate identification, quantification	Experimental conditions (especially the velocity of the carrier gas) should be continuously monitored and kept stable	U.S. EPA 1995; Hutchins and Wilson 1994

Table 1 (Contd.)

Name	Short description	Parameter measured	Applicable to	Advantages	Constraints	Reference
Gas chromatography/ mass spectroscopy	In the MS chamber, compounds separated by GC are bombarded by electrons and broken down into characteristic fragments called ions. Compounds are identified on the basis of the electrical current required to ionize the fragments. A sensor accumulating data on electrical current required to ionize that mass detects the mass of charged fragments (i.e., their molecular mass). The relative intensity of this current over all the different masses recorded for a particular compound generates its mass spectrum. The pattern of fragmentation ions in a mass spectrum has a characteristic appearance used to distinguish one compound from another. In addition, the intensity of the current recorded for one characteristic ion over time gives rise to its mass chromatogram, which is used to quantify the concentration of the analyte as it elutes from the gas chromatograph. This characteristic ion is called the quantification ion. The mass chromatograms for all ions detected in a sample can be superimposed onto a reconstructed ion chromatogram (RIC), also called a total ion chromatogram. The RIC is a graphic display of the total ionization current resulting from all mass fragments of all compounds detected throughout the analysis	Contaminant identification and determination	Identification of compounds eluted from a GC column	GC/MS enables positive identification. The RIC can be compared with the chromatograms produced by other detectors and provides an indication of the relative composition of components in the sample mixture analyzed by GC/MS	Expensive equipment, complicated experimental procedure	U.S. EPA 1995
Gas chromatography/ flame ionization detection	The flame ionization detector (FID) responds to ions produced by the burning of compounds (separated by gas chromatography) in a H_2/air flame. FID response depends on the number of ions produced by a compound. Since this varies considerably between compound classes, FID response factors vary correspondingly	Contaminant identification and determination	Identification of almost all organic compounds, after their elution from a GC column	FID response is linear in a wide range of concentrations and not influenced by temperature changes	Carbon compounds detected must be oxidizable. A few organic compounds (e.g. formic acid, acetaldehyde) exhibit poor sensitivity	Hatziioannou 1989; Prince et al. 1994; Karazek and Clement 1988; Sveum et al. 1994
Luminescence techniques	Bioluminescence is a natural phenomenon associated with several microorganisms. Reporter gene cassettes, comprised of luciferase structural genes and contaminant-specific promoter sequences, are inserted into a bacterial host. Their expression is induced by the contaminant hydrocarbon of interest. Light emitted by such systems can be measured and used to monitor bacterial metabolic activity using the specific contaminant	Microbial activity	Determination of the aromatic content of a complex mixture of hydrocarbons. The aromatic content is important because of its toxic nature and stability against weathering. Provides preliminary assessment of contaminant bioavailability for biodegradation	High sensitivity and reasonable selectivity. Direct examination of oil mixtures in water is possible under favorable circumstances. Does not require the disruption of cellular activities or structures; provides real-time data on cellular catabolic activities in vivo	Severe and not quantifiable quenching of light emission in soil slurries compared to artificial, soil-free media. Some bacteria do not exhibit bioluminescence. Limited application in multi-species oil-degrading microbial communities and under field conditions of poorly controlled environmental parameters	Grigson et al. 1985; Sanseverino et al. 1994; Hastings et al. 1985; Heitzer et al. 1992

Fluorescence analysis	During fluorescence analysis of a compound, excitation and emission spectra are recorded. These two spectra can be corrected through appropriate modifications, and used for the identification of the compound	Contaminant identification and determination	Oil fingerprinting	This technique has become a part of a basic and routine analytical procedure, because of the considerable success in identification following oil spills in coastal waters. This procedure involves the use of TLC, IR and GC measurements	A conventional fluorescence spectrum merely represents one section across a three-dimensional matrix determined by the dependence of intensity on the twin wavelength parameters of emission and excitation. The subjectivity imposed by the choice of a single excitation wavelength can lead to the exclusion of valuable conventional fluorescence spectra. Although valuable as a rapid means of discrimination between some suspect sources this method is severely limited in its ability to differentiate between hydrocarbon mixtures of similar composition	Grigson et al. 1985; Hatziioannou 1989
Use of internal petroleum biomarkers	This analytical approach is used to quantify the extent of oil weathering from biological or physical processes. Provided that all concentration data are expressed on an oil-weight basis, the increase in the internal biomarker concentration relative to the concentration in the source oil is proportional to the amount of oil lost through weathering and biodegradation. The method is gravimetric and relies on GC/FID and GC/MS to determine total oil or specific analyte concentrations	Contaminant identification and determination	Both field and laboratory studies of petroleum and refined products. Provides quantitative information about the degradation of petroleum in highly variable environments	Field data indicate that the use of an internal chemical indicator reduces spatial variability of oil data when compared to other mass-balance approaches. This allows effective degradation monitoring by reducing the number of samples required	The application of an internal chemical indicator to monitor oil weathering or degradation is based on the following assumptions: (1) the source of oil contamination has been identified and is primarily a single source, (2) the chemical indicator is not formed or degraded during biodegradation, and (3) the extraction efficiency of the chemical marker is the same as the rest of the oil	Douglas et al. 1994; Prince et al. 1994
TPH/infrared spectroscopy – TPH/ gas chromatography	Total petroleum hydrocarbons/IR spectroscopy (EPA 418.1 modified for soils; U.S. EPA 1986, 1989) is the method where an oiled sample is solvent-extracted. The total mass of dissolved hydrocarbon is subsequently quantified by comparing the infrared absorption of the extraction liquid against that of a defined hydrocarbon mixture. Gas chromatographic methods for petroleum hydrocarbon analysis include the American Society for Testing and Materials (ASTM) method 3328.78 (ASTM 1989) and EPA modified method 8015 (US EPA 1986, 1989). Both methods use GC/FID to measure petroleum hydrocarbons	Contaminant identification and determination	Measurements of total petroleum hydrocarbons (TPH) for the assessment/screening of diesel-fuel-contaminated soils, as well as for determining site closure	Low technical complexity, quick quantification	The mixture in which the diesel fuel bioremediation is being performed can affect both analytical methods. Matrix effects are site-specific. Neither analytical method can overcome these limitations. Part of the difficulty is the use of a non-compound -specific measurement to assess progress. One way to overcome this difficulty may be through the use of target or specific marker compounds	Troy and Jerger 1994

Table 2 Available microbial strains used in bioremediation

Name	Description	Supplier	Reference
HYDROBAC	Bacterial preparation specific for petroleum hydrocarbon materials	Polybac Corporation	Huesemann and Moore 1994
Pseudomonas, *Rhodococcus*, *Arthrobacter*	Biosurfactant-producing bacteria		Hunt et al. 1994
Pseudomonas putida	Naphthalene-degrading bacteria	ATCC30015	Churchill et al. 1995
P. oleovorans		ATCC29347	
Unknown		ATCC15075	
Acinetobacter calcoaceticus MM5	Bacterial species		Marin et al. 1995
Pseudomonas fluorescens 2a	Bacterial species	TsMPM B2556	Kozhanova 1991
Alteromonas, *Pseudomonas*, *Deleya*, *Moraxella*, *Bacillus*, *Flavobacterium*, *Micrococcus*, *Mycobacterium* and *Vibrio*, or mixture of above	Marine aerobic bacteria		Kaushanskii et al. 1994
Candida sp.	Fungus	FERM P-11023	Anonymous 1995
S1EW1		FERM P-13871	
S2EW1		FERM P-13872	
S1OW5		FERM P-13873	
Candida tropicalis VSB-637 and *Mycococcus lactis* VSB-D5 (pair)	Bacterial and fungal species		Bitteeva et al. 1994
Mycococcus lactis VSB-574 and VSB-D5 (pair)			
Acinetobacter oleovorum subsp. *paraphinicum* VSB-576 and *Candida guilliermondii* subsp. *paraphinicum* VSB-638 (pair)[a]	Bacterial and fungal species		Bitteeva et al. 1994
Candida sp. M23-2	Fungus	FERM P-13540	Anonymous 1994a
Trichoderma sp. AP-5	Fungus	FERM P-13541	Anonymous 1994b
Rhodococcus erythropolis	Bacterial species	BKM Ac-1339D	Anonymous 1993a
BB-232	Petroleum-degrading bacterium		Cubitto and Cabezali 1994
Bacillus sp.	Petroleum-degrading bacterium	BKPM B-5467	Anonymous 1992
Pseudomonas putida, and *Geotrichum candidum*	Mixed bacteria/fungi culture	CCM 4307 CCM 8170	Anonymous 1993c
Pseudomonas alkaligenes or *Alcaligenes* sp. ER-RL3	Bacterial species	NCIMB 40464	Anonymous 1993b
Pseudomonas sp. ER-RL4		NCIMB 40465	
Gluconobacter sp. ER-RT		NCIMB 40466	
Acinetobacter calcoaceticus ER-RLD		NCIMB 40506	
Acinetobacter calcoaceticus ER-RLX		NCIMB 40507	

Table 3 In situ treatments

Treatment	Description	Applicable to	Advantages	Constraints	Reference
Biostimulation	Biostimulation involves the addition of oxygen, water and mineral nutrients (usually combinations of nitrogen, phosphorus, and trace metals)	Groundwater, soils	Acceleration by as much as 100-fold of the reproduction of organisms already present at the site	Nutrient application requires concurrent ecological monitoring studies. Although dilution, tidal mixing etc. should minimize the potential for adverse ecological effects, certain components of the fertilizer could be toxic to some species. In addition, algal blooms could occur as a result of the sudden availability of nitrogen and phosphorus	Orzech et al. 1991; U.S. EPA 1989
Bioventing	This remediation technique combines conventional advective soil venting with biodegradation. The soil-venting phase of the process takes advantage of the highly volatile nature of the components present in the hydrocarbon mixture. In the biodegradation phase, the less volatile high-molecular-mass hydrocarbon compounds are broken down and the more volatile organics removed during conventional venting	Soils	Addresses full range of petroleum hydrocarbons. Is among the most effective methods of supplying indigenous microorganisms with enough oxygen to support degradation of hydrocarbon contaminants	A shallow water table prevents diffusion of gases and makes application of bioventing less feasible. Furthermore, the solid matrix must allow for sufficient air movement	Van Eyk 1994; Reisinger et al. 1994
Bioaugmentation	This method involves the direct application of microorganisms originating from (a) the remediation site (b) an off-site vendor (c) genetic engineering. The microorganisms have been cultured and adapted, while their degrading ability can be enhanced for specific contaminants and site conditions	Groundwater, soils	One of the most effective bioremediation techniques	The possibility of adverse ecological effects from the introduction of off-site or genetically engineered microorganisms should be examined and minimized	Orzech et al. 1991; Leavitt and Brown 1994
Surfactants	Synthetic or biogenic substances are used to increase the aqueous solubility of solid hydrocarbons, and emulsify liquid hydrocarbons	Solid and liquid aliphatic and aromatic hydrocarbons (including hydrophobic organic compounds)	Enhancement of contaminant accessibility to microorganisms, nutrients, and possibly oxygen. Improvement of the bioremedial efficiency	Surfactant use is still controversial. Synthetic surfactants may adversely affect the permeability of the microbial cell membrane, thus reducing or eliminating the biodegradative potential of indigenous microorganisms. Surfactant injection must be carefully controlled to be ecologically acceptable and to offer more economical and physical advantages than disadvantages	Ducreux et al. 1994; Churchill et al. 1995

Table 3 (Contd.)

Treatment	Description	Applicable to	Advantages	Constraints	Reference
Hydrogen peroxide	Hydrogen peroxide can be used as an efficient, alternative oxygen source to enhance in situ biodegradation	Biodegradation of aromatic compounds and volatile fatty acids. Subsurface applications	Provides oxygen at a rate up to two orders of magnitude faster than diffusers (systems to saturate injected water with air). The growth of aerobic bacteria in general and hydrocarbon-degrading bacteria in particular, as well as the breakdown of gasoline are significantly enhanced by the addition of H_2O_2	Although H_2O_2 has been used successfully in a number of cases, problems, including too rapid decomposition, gas blockage, and ineffectiveness, have been encountered elsewhere	Lu 1994a; Brown and Norris
Nitrate	Nitrate is used as an alternative electron acceptor, instead of aeration of the injected water or amendment with H_2O_2	Subsurface applications	Nitrate is relatively inexpensive, highly soluble in water, does not adsorb to soil matrices, and does not decompose. Thus it can be readily distributed within an aquifer	Nitrate concentrations in groundwater typically are limited by regulatory standards to 46 mg/l. Another disadvantage is that nitrate is effective for fewer classes of compounds than oxygen. Nitrate-utilizing bacteria do not degrade aliphatic compounds. Benzene is not readily degraded under denitrifying conditions	Hutchins and Wilson 1994; Brown and Norris 1994b; Battermann et al. 1994
Fertilizer application (water-soluble or oleophilic)	Fertilizer application stimulates microbial metabolism by supplying the indigenous oil-degrading microorganisms with nutrients (N, P, K, etc.)	Soil, groundwater, sediments	Acceleration of the natural biodegradation process, especially in sites with low nutrient content	A toxicology assessment is required to evaluate the possibility of harmful biological effects of excessive nutrient application	Prince et al. 1994; Sveum et al. 1994

co-oxidize these compounds. However, because fungi can not further metabolize the products of co-oxidation, mixed cultures with bacteria are required for complete mineralization of the organic contaminant (Bouwer and Zehnder 1993).

The origin of the organisms to be used in bioremediation is debatable. Indigenous microorganisms isolated from the contaminated site are certainly adapted to the climatic, physicochemical and nutrient conditions prevalent therein. Bioremediation by these microorganisms is expected to proceed at increased rates after nutrient addition and/or seeding with enriched microorganism cultures. However, these microbial communities may not include the full range of species or enzymes required for effective oil biodegradation. Commercially available preparations of oil-biodegrading microorganisms usually include many species and have an increased potential to degrade various oil components effectively. Suppliers claim that these mixtures can be custom-made for the specific oil or environmental conditions and can also be easily produced for emergency situations.

The commonest methods of in situ hydrocarbon bioremediation are tabulated in Table 3. The addition of N_2, P or oxygen in various forms is thought to be sufficient to enhance the growth of microbes capable of utilizing carbon in the form of petroleum hydrocarbons (Alexander 1991). However, bioremediation is not a panacea against organic contamination. The spectacular results of laboratory experiments cannot always be transferred directly to the field. Many compounds that are easily metabolized in vitro are often not broken down efficiently in contaminated soils and aquifers. This is probably due to reduced contaminant bioavailability caused by adsorption on soil particles or solution in nonaqueous-phase liquids (Alexander 1991). Another bioremediation-restricting factor is the patchy distribution of bacterial populations in the soil. This natural heterogeneity of environmental conditions on the microhabitat scale results in localization of the hydrocarbon-degrading bacteria.

In contrast to the wide public acceptance of bioremediation using indigenous microorganisms, the public and the scientific community are somewhat reluctant to accept the method of seeding with introduced microorganisms. The main reasons for this (Atlas 1977) are (1) the lack of controlled experiments demonstrating superior performance of introduced microorganisms compared to indigenous ones, (2) the time lag between microorganism application and hydrocarbon breakdown, (3) the lack of information on microorganism pathogenicity to humans, genetic stability and toxicity of metabolic byproducts, (4) the large quantities of microorganisms required for frequent application to the contaminated site, (5) the logistics of culture preparation and mixing just prior to application on site, and (6) the fate of these microorganisms once they have completed their role in bioremediation.

Future research should be directed towards the improvement of existing and the devising of innovative methods of bioremediation. Attention should be paid to the factors governing bioavailability of organic contaminants and methods to increase availability and microorganism activity, monitoring of bioremediation, the ecology and fate of introduced microorganisms, the transfer of laboratory findings to the field, the elucidation of fertilizer action, the discovery of new, more efficient fertilizers and ways of application, the coupling of major biochemical cycles, such as nitrogen and phosphorus, with bioremediation cases, and the effects of other environmental factors (e.g. temperature and salinity fluctuations, wave action, oxygen availability, nitrogen-fixing bacteria, etc.); most importantly, all of the above should be investigated in the field under realistic conditions.

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