

Application of bioremediation technology in the environment contaminated with petroleum hydrocarbon

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Abstract The demand for petroleum and petroleum products is increasing day by day, while oil spills and improper discharge of industrial wastes contributes to the rising contamination of the environment with petroleum hydrocarbon. Petroleum hydrocarbon spills cause various carcinogenic and neurotoxic effects, and thus effective treatment strategies are required. The various physical and chemical methods currently in use are costly and leave toxic residues in the environment. In contrast, bioremediation is a promising technology in the treatment of petroleum hydrocarbon contamination because of its high effectiveness, lower cost and environmental synergy. Here, we review the biodegradation of petroleum hydrocarbon and various factors influencing this process.

Keywords Petroleum hydrocarbon · Bioremediation · Environment · Pollution

Introduction

As the world's population continues to grow, the demand for petroleum and petroleum products increases vigorously, while

oil spills and the improper discharge of industrial wastes contribute to pollution of the environment (Hernandez et al. 1998; Gupta and Mahapatra 2003; Strong and Burgess 2008). In 1971, in Whitmarsh Township, Pennsylvania, a gasoline pipeline broke and released several hundred thousand gallons of high-octane gasoline. It was estimated that 100,000 gallons (379,000 L) of gasoline seeped into the underlying dolomitic aquifer and the dissolved petroleum hydrocarbons migrated to a nearby municipal water supply well. It was recently proposed that at the time, that ground water system might have contained petroleum hydrocarbon-degrading microorganisms (Litchfield and Clark 1973). Another major oil spill occurred in 1989, in Prince William Sound, Alaska, in which the oil tanker Exxon Valdez wrecked and spilled more than 200,000 barrels of crude oil into the water (Hagar 1989). Several other major oil spills have occurred in Texas, Rhode Island, and the Delaware Bay (Anonymous 1989). These spills have attracted the attention of researchers examining the problem of hydrocarbon contamination and remediation of water and land environments. The amount of natural crude oil seepage was estimated to be 600,000 tons per year, with a range of uncertainty of 200,000 metric tons per year (Kvenvolden and Cooper 2003). Thus, control and treatment strategies to reduce the hazardous effects of oil pollution are needed. However, conventional physical-chemical treatments have high costs and can generate residues that are toxic to the biota. Applying high efficiency and low cost bioremediation processes represent an extremely important way of recovering contaminated areas among several other clean up techniques (Bidoia et al. 2010). The success of bioremediation efforts in the cleanup of the Exxon Valdez oil spill created tremendous interest in the potential of biodegradation and bioremediation technology. This was one of the first instances where bioremediation was actively considered as a remedial strategy to clean up a petroleum-contaminated ground system (Chapelle

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1999). Bioremediation, a process that utilizes the natural capability of microorganisms to degrade toxic waste, is emerging as a promising technology for the treatment of soil and groundwater contamination (Milic et al. 2009). This review contains a broad and updated overview on bioremediation of hydrocarbon contaminated-environments, microbial degradation of petroleum hydrocarbons, and various physical, chemical, and biological factors influencing biodegradation.

Petroleum hydrocarbon

Petroleum was first obtained by the Chinese in pre-Christian times and has been known for several years to occur in surface seepage (Okoh 2006). The modern petroleum industry had its beginning in Romania and in a Pennsylvania well sunk by Colonel E. A. Drake in 1859 (Alloway and Ayres 1993). Petroleum is a naturally occurring, oily, flammable liquid that is present beneath the surface of the earth. It is a mixture of hydrocarbons and elements like sulphur, oxygen, and nitrogen. In the refining industry, petroleum refers only to crude oil (Van 1997). Crude oil originates in naturally occurring geological deposits formed from the organic decomposition of ancient plants and animals under high temperatures and pressure. Crude oil is principally composed of liquid hydrocarbons (HCs) having four or more carbon atoms (Manning and Thompson 1991; Van 1997). Alkanes, cycloalkanes, and aromatics are the three principle classes of hydrocarbons present in petroleum (Strausz and Lown 2003). Alkenes and alkynes are present only in trace quantities (Van 1997; Potter and Simmons 1998). These compounds can be categorized into four simple fractions: saturated (or alkanes); aromatics, including compounds such as benzene, toluene, ethylbenzene and xylenes; (BTEX) and polyaromatic hydrocarbons (PAHs); resins, consisting of compounds containing nitrogen, sulphur, and oxygen that are dissolved in oil; and asphaltenes, which are large and complex molecules that are colloiddally dispersed in oil. The physical properties and exact chemical composition of crude oil varies from one locality to another. In most soil and sediments, hydrocarbons are present in low concentrations (Rosenberg et al. 1992). Hydrocarbon contamination of surface and subsurface soil can result from accidental spills, equipment failure, and leaks in underground facilities, such as underground storage tanks, pipeline, or earthen pits (flare pits, sumps). At abandoned well sites, the source of PHCs in soils is generally from spills at well centers or from the disposal of drilling wastes in sumps or flare pits that are used to store or burn fluids produced at the sites. Soil contamination with hydrocarbons causes extensive damage of the local system since bioaccumulation of pollutants in animals and plant tissue may cause death or mutations (Alvarez and Vogel 1991).

Hazards of petroleum hydrocarbon contamination

The primary effects of exposure to petroleum hydrocarbons are central nervous system depression and polyneuropathy. Studies have shown that repeated exposure of laboratory animals to high concentrations of whole gasoline vapors at 67,262 and 2,056 ppm caused kidney damage and kidney cancer in rats and liver cancer in mice. The International Agency for Research on Cancer (IARC) has listed gasoline as possibly carcinogenic (2B) to humans (IARC 2000; Mortelmans et al. 1986). Among the components of gasoline, N-heptane and cyclohexane are known to cause narcosis and irritation of the eyes and mucous membranes. Cyclohexane has been reported to cause liver and kidney changes in rabbits, and n-heptane has been reported to cause polyneuritis following prolonged exposure. The IARC, the National Toxicology program (NTP) and the Occupational Safety and Health Administration (OSHA) all classify benzene as a human carcinogen. Studies have shown that the effects of benzene exposure can include leukemia at concentrations as low as 1 ppm. Methyl tertiary butyl ester (MTBE) is another potential human carcinogen, with studies showing that this component is a carcinogen in animals. It does not appear to readily biodegrade in the environment and persists both in aerobic and anaerobic conditions; it has an unpleasant taste and odor even at low concentrations. Other compounds of interest in petroleum are benzene toluene, ethyl benzene, and xylenes (BTEX). The isomers of trimethylbenzene may cause nervousness, tension, anxiety and asthmatic bronchitis. n-Hexane is known to cause the polyneuropathy (peripheral nerve damage) after repeated and prolonged exposure, while other hexanes show narcotic effects at 1,000 ppm and are not metabolized like n-hexane. Toluene can cause impairment of coordination and momentary loss of memory at exposures of 200–500 ppm, and causes palpitations at 500–1,500 ppm. Crude oil is not considered a hazardous waste (petroleum is considered a hazardous material), but crude oil coats and kills sea life and mucks up the surrounding beaches, rocks, trees, etc. (Viswanathan 1999).

Remediation processes

Physical and chemical processes are usually used in the remediation of contaminated areas; recovery, dispersion, dilution, sorption, volatilization and abiotic transformations are all important means of hydrocarbon elimination. However, these types of treatment systems require heavy machinery and the environmental consequences of removing pollutants with these techniques may result in massive air pollution (Matsumiya and Kubo 2007). Allaying high efficiency and low costs, bioremediation processes represent an extremely important alternative

for rehabilitating oil-contaminated areas compared to other conventional clean up techniques (Bhupathiraju et al. 2002). Remediation by physical treatments has shown that even though the soil is unaffected or unmodified by these treatments, the costs are high and thus not applicable on a large scale. In spite of this, biodegradation is most often the primary mechanism for contaminant clean-up (Alexander 1999; Margesin and Schinner 2001) and for the remediation of hydrocarbon-contaminated sites, biological technologies are a widely used, cost-efficient and sustainable approach (Leeson and Hinchee 1997).

Bioremediation

Bioremediation, which utilizes the biodegradative potentials of organisms or their attributes, is an effective technology that can be used to accomplish both effective detoxification and volume reduction. It is useful in the recovery of sites contaminated with oil and hazardous wastes (Caplan 1993). Moreover, bioremediation technology is believed to be non-invasive and relatively cost-effective (April et al. 2000). In some cases, it may not require more than the addition of some degradation enhancers (surfactants, nutrients, etc.) to the polluted system. It could end up being the most reliable and probably least expensive option for exploitation in solving some chemical pollution problems (Mesarch et al. 2000).

Physical factors affecting biodegradation of petroleum hydrocarbon

Knowledge of the characters and parameters that affect the microbial biodegradation of pollutants is required for the successful application of bioremediation technology. A number of limiting factors have been recognized that affect the biodegradation of petroleum hydrocarbons, many of which have been discussed by Brusseau (1998).

Physical and chemical composition of hydrocarbon

The susceptibility of hydrocarbons to biodegradation is determined by the structure and molecular weight of the hydrocarbon molecule. Aliphatic hydrocarbons are degraded and assimilated by a wide range of microorganisms. n-alkanes of intermediate chain length (C₁₀–C₂₄) are degraded most rapidly. Short-chain alkanes (less than C₉) are toxic to many microorganisms but being volatile, are generally lost rapidly in the atmosphere. Long-chain alkanes are generally resistant to biodegradation. Branching in general reduces the rate of biodegradation. Aromatics may be partly oxidized but are metabolized by only a few bacteria. Aromatic compounds,

especially polyaromatic hydrocarbons (PAHs), are degraded slowly. Alicyclic compounds can be degraded via a process known as cometabolism. The bioavailability of hydrocarbons, which is largely a function of concentration and physical state, as well as their hydrophobicity, sorption onto soil particles, volatilization and solubility greatly affect the extent of their biodegradation. Hydrocarbons differ in their susceptibility to microbial attack and, in the past, have generally been ranked in the following order of decreasing susceptibility: n-alkanes > branched alkanes > low-molecular-weight aromatics > cyclic alkanes (Perry 1984). Biodegradation rates have been shown to be highest for the saturated, followed by the light aromatics, with high-molecular-weight aromatics and polar compounds exhibiting extremely low rates of degradation (Jobson et al. 1972; Walker et al. 1976; Fusey and Oudot 1984). This pattern is not universal, however, as Cooney et al. (1985) reported observing greater degradation of naphthalene instead of hexadecane in water-sediment mixtures from a freshwater lake and extensive biodegradation of alkylaromatics in marine sediments prior to detectable changes in the n-alkane profile of crude oil (Jones et al. 1983). Fedorak and Westlake (1981) also reported a more rapid attack of aromatic hydrocarbons during the degradation of crude oil by marine microbial populations from a pristine site and a commercial harbor.

Temperature

Among physical factors, temperature plays an important role in the biodegradation of hydrocarbons by directly affecting the chemistry of the pollutants as well as affecting the physiology and diversity of the microbial flora. Atlas (1975) found that at low temperatures, the viscosity of the oil increased, while the volatility of the toxic low molecular weight hydrocarbons were reduced, delaying the onset of biodegradation. Temperature also affects the solubility of hydrocarbons (Foght et al. 1996). At low temperature, hydrocarbon water solubility increases. Some previously reported data on the solubility of hydrocarbons in water are summarized in Table 1 (Bahram 1969) and 2 (<http://esc.syrres.com/efdb/chemfate.htm>). Although hydrocarbon biodegradation can occur over a wide range of temperatures, rates of degradation are generally observed to decrease with decreasing temperature; this is believed to be a result primarily of decreased rates of enzymatic activity, or the “Q₁₀” effect (Atlas and Bartha 1972; Gibbs et al. 1975). The highest degradation rates generally occur in the range of 30–40°C in soil environments, 20–30°C in some freshwater environments and 15–20°C in marine environments (Bartha and Bossert 1984; Cooney 1984). Venosa and Zhu (2003) reported that ambient temperatures of the environment affected both the properties of spilled oil and the activity of the microorganisms. Significant biodegradation of hydrocarbons have been reported in psychrophilic environments in temperate regions

Table 1 Solubility of methane, ethane, propane and n-butane in water at 100°F and pressure up to 1,000 psi

Pressure psi	Solubility ($10^3 \times$ mole fraction of hydrocarbon in water)			
	CH ₄ (Culberson 1951)	C ₂ H ₆ (Culberson 1951)	C ₃ H ₈ (Kobayashi 1951)	n-C ₄ H ₁₀
200	0.273	0.328	0.204	0.002
400	0.528	0.534	0.208	0.063
600	0.760	0.667	0.211	0.064
800	0.986	0.749	0.214	0.064
1000	1.199	0.793	0.217	0.065

(Pelletier et al. 2004; Delille et al. 2004), however, the bulk of information that exists on hydrocarbon degradation details the activities of mesophiles (Yumoto et al. 2002; Pelletier et al. 2004; Delille et al. 2004). Documented research on the environmental consequences of terrestrial spills in cold regions remains scarce, even though petroleum contamination is recognized as a significant threat to polar environments. Full-scale in situ remediation of petroleum contaminated soils has not yet been used in Antarctica for example, partly because it has long been assumed that air and soil temperatures are too low for effective biodegradation (Delille et al. 2004). Climate and season would be expected to select for different populations of hydrocarbon-utilizing microorganisms which are adapted to ambient temperatures. Colwell et al. (1978) reported extensive degradation of Metula crude oil by mixed cultures of marine bacteria at 3 °C, and Huddleston and Cresswell (1976) observed petroleum biodegradation in soil at -1.1 °C. By contrast, only negligible degradation of oil was exhibited in frozen tundra soil (Atlas et al. 1976) and in Arctic marine ice (Atlas et al. 1978). Low winter temperatures were the limiting factor for the biodegradation of polyaromatic hydrocarbons in estuarine sediment (Shiaris 1989) and of a variety of hydrocarbons in freshwater lakes (Cooney et al. 1985).

Oxygen

Hydrocarbons, being highly reduced substrates, require an electron acceptor, with molecular oxygen being most common. Though most studies have shown the biodegradation of

hydrocarbons to be an aerobic process, anaerobic biodegradation of hydrocarbons has also been reported. In the absence of molecular oxygen, nitrate, iron, bicarbonate, nitrous oxide and sulfate have been shown to act as alternate electron acceptors during hydrocarbon degradation. Oxygen is typically the limiting factor in aerobic bioremediation at many sites. The degradation of petroleum hydrocarbons occurs much faster under aerobic conditions compared to anaerobic conditions. Therefore, the addition of oxygen can significantly increase the remediation rate. The initial steps in the catabolism of aliphatic (Singer and Finnerty 1984a), cyclic (Perry 1984), and aromatic (Cerniglia 1984a) hydrocarbons by bacteria and fungi involve the oxidation of the substrate by oxygenases, for which molecular oxygen is required. The concentration of oxygen has been identified as the rate-limiting variable in the biodegradation of petroleum in soil (Von Wedel et al. 1988) and of gasoline in groundwater (Jamison et al. 1975). The availability of oxygen in soils is dependent upon rates of microbial oxygen consumption, the type of soil, whether the soil is waterlogged, and the presence of utilizable substrates which can lead to oxygen depletion (Bossert and Bartha 1984). Anaerobic degradation of petroleum hydrocarbons by microorganisms has been shown in some studies to occur only at negligible rates (Bailey et al. 1973; Jamison et al. 1975; Ward and Brock 1978a), and its ecological significance has been generally considered to be minor (Atlas 1981; Bossert and Bartha 1984; Cooney 1984; Floodgate 1984). However, the microbial degradation of oxidized aromatic compounds such as benzoate (Tarvin and Buswell 1934) and of halogenated aromatic compounds such as the halobenzoates (Sufliata et al. 1982), chlorophenols (Boyd and Shelton 1984), and polychlorinated biphenyls (Chen et al. 1988) has been shown to occur under anaerobic conditions. Recent evidence also indicates that microbial consortia from soil and sludge are capable of metabolizing unsubstituted and alkyl-substituted aromatics, including benzene, toluene, xylene, 1,3-dimethylbenzene, acenaphthene, and naphthalene, in the absence of molecular oxygen (Grbic-Galic and Vogel 1987; Mihelcic and Luthy 1988a, b; Zeyer et al. 1986). Anaerobic degradation of aliphatic hydrocarbon has also been reported and has been linked to denitrification (Bregnard et al. 1997; Chayabutra and Ju 2000; Wilkes et al. 2002), sulfate

Table 2 Solubility of common petroleum fuel constituents

Constituent	Effective solubility in water ^a (mg/L) (25 °C)
Benzene	24 to 95
Toluene	12 to 60
Ethylbenzene	8 to 33
m-Xylene	3 to 13
o-Xylene	3 to 14
p-Xylene	4 to 16
MTBE	5,600 to 8,760

^aRecommended values from CHEMFATE Database, Syracuse Research Corp., <http://esc.syrres.com/efdb/chemfate.htm>

reduction (Coates et al. 1997; Kropp et al. 2000; Rueter et al. 1994; So and Young 1999) and methanogenesis (Anderson and Lovley 2000; Zengler et al. 1999).

Nutrients

For successful biodegradation of hydrocarbon pollutants, nutrients are very important ingredients, especially nitrogen, phosphorus, and in some cases iron (Cooney 1984). Some of these nutrients could become limiting factors, thus affecting the biodegradation processes. Atlas (1985) reported that when a major oil spill occurred in marine and freshwater environments, the supply of carbon was significantly increased and the availability of nitrogen and phosphorus generally became the limiting factor for oil degradation. To maintain an adequate microbial population, nutrients need to be supplemented with time in sufficient quantities in the aquifer. Depending on the constituents and microbes involved in the biodegradation process, carbon:nitrogen:phosphorus ratios necessary for enhancing biodegradation fall in the range of 100:10:1 to 100:1:0.5. To avoid overapplication of nitrogen and phosphorus, it is important to understand how much carbon can be metabolized based on oxygen-limiting conditions. Nitrogen and phosphorus should be added based on the amount of carbon that can be metabolized at any given time compared to the total average concentration of carbon (i.e., petroleum contamination) in the subsurface. For example, in a full scale operation to treat the area, a net 0.6 lb per hour of pure oxygen is introduced and is assumed to be completely utilized; during aerobic microbial activity approximately 0.17 lb per hour (4 lb per day) of hydrocarbon is theoretically oxidized by microbes (using a 3.5:1 oxygen:hydrocarbon stoichiometric ratio). Then, using the theoretically optimal ratio range of 100:10:1 to 100:1:0.5 C:N:P for this example, 0.4–0.04 lb per day of nitrogen and 0.04–0.02 lb per day of phosphorus may need to be added to the treatment area to keep up with the estimated carbon metabolism rate (http://www.epa.gov/oust/pubs/tum_ch12.pdf). In nutrient limited wastewaters, a BOD:N:P ratio of 100:5:1 is often used as a benchmark for nutrient addition. A study in a simulated aerated lagoon was performed by Slade et al. (2011) to check the effect of nitrogen at different concentration levels while keeping the phosphorus concentration constant (BOD:N of 100:0; 100:1.3; 100:1.8; 100:2.7 and 100:4.9). A synthetic wastewater was prepared in which methanol, glucose and acetate acted as the combined carbon source, ammonium chloride was used for a nitrogen source, and dipotassium hydrogen phosphate was used as a phosphorus source. When nitrogen was not supplemented, dispersed growth was observed. Increased filamentous growth was observed by increasing nitrogen levels, with a marked change in filamentous species occurring between a BOD:N ratio of 100:1.8 and 100:2.7. Nitrogen fixation

occurred at a BOD:N ratio of 100:0; 100:1.3 and 100:1.8, with nitrogen loss at BOD:N ratios of 100:2.7 and 100:4.9. At a BOD:N ratio of 100:4.9, ammonium discharge was significantly greater (1.8 mg/L) than at the lower nitrogen levels (0.04–0.18 mg/L). Phosphorus behavior was more variable, however significantly more phosphorus was discharged at the lowest nitrogen level than at the highest ($p < 0.05$). Based on readily available nitrogen, the BOD:N ratio at which nitrogen fixation no longer occurred was around 100:1.9. In marine environments, it was found to be more pronounced due to low levels of nitrogen and phosphorus in seawater (Floodgate 1984). Freshwater wetlands are typically considered to be nutrient deficient due to heavy demands of nutrients by the plants (Mitsch and Gosselink 1993). Therefore, additions of nutrients were necessary to enhance the biodegradation of oil pollutants (Choi et al. 2002; Kim et al. 2005). On the other hand, excessive nutrient concentrations can also inhibit the biodegradation activity (Chaillan et al. 2006). Several authors have reported the negative effects of high NPK levels on the biodegradation of hydrocarbons (Oudot et al. 1998), especially on aromatics (Carmichael and Pfaender 1997). An experiment was set up by Oudot et al. (1998) in the mid-tide sediments of an estuarine environment in the bay of Brest, France to study the influence of the addition of a slow-release fertilizer on the biodegradation rate of crude oil for 9 months. The weathering of total oil and fractions was monitored relative to the internal conservative biomarker 17 α (H), 21 β (H)-30-norhopane by computerized capillary gas chromatography. The results of biodegradation rates for total oil, aliphatics, cycloalkanes and aromatics were respectively 40 \pm 7, 83 \pm 6, 49 \pm 10 and 55 \pm 18 %. The resins and asphaltenes were not degraded. There was no significant difference in biodegradation rates observed between fertilized and non-fertilized plots, which was attributed to the high background level of N and P. It was estimated that if background level of N in the interstitial pore water of the sediment is ≥ 100 μ moles/liter then bioremediation through fertilization may be of limited use only. Another study on biodegradation of PAH was carried out by Carmichael and Pfaender (1997) to examine the effects of several bioremediation stimulants, including potential metabolism pathway inducers, inorganic/organic nutrients, and surfactants on the metabolism of phenanthrene and pyrene, as well as the population dynamics of PAH-degrading microorganisms in five soils with differing background PAH concentrations, exposure histories and physical properties. Increases in the populations of heterotrophic microorganisms was observed with many of the supplements, as measured by plate counts, but no increase in the populations of phenanthrene-degrading microorganisms was observed, as measured by the [14 C]-PAH mineralization MPN analysis or cellular incorporation of [14 C]-PAH. The results suggested that the PAH-degrading community at each site may be unique in their response to materials added in an attempt to stimulate PAH degradation. The

characteristics of the site, including exposure history, soil type, and temporal variation may all influence their response. The effectiveness of fertilizers for the crude oil bioremediation in subarctic intertidal sediments was studied by Pelletier et al (2004). The use of poultry manure as organic fertilizer in contaminated soil was also reported (Okolo et al. 2005), and biodegradation was found to be enhanced in the presence of poultry manure alone. Maki et al. (2005) reported that photo-oxidation increased the biodegradability of petroleum hydrocarbons by increasing their bioavailability and thus enhancing microbial activities. Nitrogen and phosphorus may also be limiting in soils, and the acceleration of the biodegradation of crude oil or gasoline in soil and groundwater by the addition of urea-phosphate, N-P-K fertilizers, and ammonium and phosphate salts has been demonstrated in several studies (Dibble and Bartha 1979; Jamison et al. 1975; Jobson et al. 1974; Verstraete et al. 1976). Other investigators observed no increase in biodegradation rates (Lehtomaki and Niemela 1975) or an increase only after a delay of several months to a year (Odu 1978), (Raymond et al. 1976) when fertilizer amendments were used. These seemingly contradictory results have been attributed by Bossert and Bartha (1984) to the variable and complex composition of soils and to other factors such as nitrogen reserves and the presence of nitrogen-fixing bacteria.

Acidity or alkalinity

The acidity and alkalinity (PH) is also a very important factor in the biodegradation of petroleum hydrocarbon. In contrast to most aquatic ecosystems, soil pH can be highly variable, ranging from 2.5 in mine spoils to 11.0 in alkaline deserts (Bossert and Bartha 1984). Most heterotrophic bacteria and fungi prefer a pH near neutral, with fungi being more tolerant of acidic conditions (Atlas 1988). Extremes in pH, as can be observed in some soils, would therefore be expected to have a negative influence on the ability of microbial populations to degrade hydrocarbons. Verstraete et al. (1976) reported a near doubling of biodegradation rates of gasoline in an acidic (pH 4.5) soil by adjusting the pH to 7.4. Rates dropped significantly, however, when the pH was further raised to 8.5. Similarly, Dibble and Bartha (1979) observed an optimal pH of 7.8 in the range 5.0 to 7.8 for the mineralization of oily sludge in soil. The pH of sediments in special environments such as salt marshes may be as low as 5.0 in some cases (Patrick and DeLaune 1977). Hambrick et al. (1980) found the rates of microbial mineralization of octadecane and naphthalene to be depressed at this pH compared with pH 6.5. Octadecane mineralization rates increased further when the pH was raised from 6.5 to 8.0, whereas naphthalene mineralization rates did not.

Soil moisture

Soil moisture is another important parameter in determining the rate of biodegradation of petroleum compounds. Microbes live in the interstitial water of soil pores and thus, a lower amount of water means a smaller number of microbes are present and the removal rate through biodegradation is slower (Dibble and Bartha 1976). In terrestrial ecosystems, hydrocarbon biodegradation may be limited by the availability of water for microbial growth and metabolism. Water potential (a_w) of soils can range from 0.0 to 0.99, in contrast to aquatic environments, in which water activity is stable at a value near 0.98 (Bossert and Bartha 1984). Atlas (1981) has suggested that tarballs deposited on beaches may represent another situation in which available water limits hydrocarbon biodegradation. Dibble and Bartha (1979) reported optimal rates of biodegradation of oil sludge in soil to be at 30–90 % water saturation. A laboratory study was conducted by Dibble and Bartha (1979) with the aim of evaluating and optimizing the environmental parameters of “landfarming,” i.e., the disposal by biodegradation in soil of oily sludges generated in the refining of crude oil and related operations. Oil sludge biodegradation was optimal at a soil water holding capacity of 30–90 %, a pH of 7.5–7.8, C:N and C:P ratios of 60:1 and 800:1, respectively, and a temperature of 20° C or above. An application rate of 5 % (wt/wt) oil sludge hydrocarbon to the soil (100,000 l/hectare) achieved a good compromise between high biodegradation rates and efficient land use, and resulted in the best overall biodegradation rate of all hydrocarbon classes. Two 100,000-liter/hectare (255 barrels per acre) or four 50,000-liter/hectare oil sludge hydrocarbon applications per growing season seem appropriate for most temperate zone disposal sites in their study. The failure to observe inhibition of degradation at the lower values was ascribed to a hydrocarbon-mediated reduction in the water holding capacity of the soil.

Bioavailability

Bioavailability is a key factor in the efficient biodegradation of pollutants. It is the amount of a substance that is physicochemically accessible to microorganisms. The application of external nonionic surfactant, e.g., the main components of oil spill dispersants, influences the alkane degradation rate (Bruheim and Eimhjelle 1998; Rahman et al. 2003). The use of surfactants in situations of oil contamination may have a stimulatory, inhibitory or neutral effect on the bacterial degradation of the oil components (Liu et al. 1995). Chemical dispersants caused ecological damage after application for the abatement of spilled oil in marine ecosystems (Smith 1968), while biosurfactants from soil or freshwater microorganisms are less toxic and partially biodegradable (Poremba et al. 1991). Commercially available surfactants,

both ionic and nonionic in nature (Laha and Luthy 1992; Thai and Maier 1992; Pennell et al. 1993), as well as biosurfactants and biosurfactant-producing bacteria have been investigated for their ability to increase bioavailability (Van Dyke et al. 1991; Volkering et al. 1993; Miller 1994). Physical disruption of soil aggregates using sonication has been reported to increase biodegradation rates effectively in a landfarm experiment. Weissenfel et al. (1992) demonstrated that soil constituents have significant impact on the bioavailability of contaminants.

Salinity

Salinity plays an important role in the degradation of hydrocarbons by microorganisms. The naphthalene mineralization rate and salinity in sediments of the Hudson river were dependent upon the ambient salinity regime, with estuarine sites exhibiting a lack of inhibition of mineralization over a wider range of salinities than was the case for the less saline upstream site (Kerr and Capone). In a study of hypersaline salt evaporation ponds, Ward and Brock (1978b) showed that rates of hydrocarbon metabolism decreased with increasing salinity in the range of 3.3–28.4 % and attributed the results to a general reduction in microbial metabolic rates.

Pressure

Pressure is another important factor in the biodegradation of hydrocarbons. The degradation of tetradecane, hexadecane, and a mixed hydrocarbon substrate by a mixed culture of deep-sea sediment bacteria was monitored at 1 atm (ca. 101 kPa) and 495 or 500 atm (ca. 50,140 or 50,650 kPa) by Schwarz et al. (1974; 1975). Colwell and Walker (1977) have suggested that oil which reaches the deep ocean environment will be degraded very slowly by microbial populations and consequently, that certain recalcitrant fractions of the oil could persist for years or decades.

Microbial degradation of petroleum hydrocarbon

In 1946, Claude E. ZoBell recognized that many microorganisms have the ability to utilize hydrocarbons as their sole source of carbon and energy. Hydrocarbons in the environment are biodegraded primarily by bacteria and fungi. Although ubiquitous in terrestrial (Jones and Edington 1968; Atlas et al. 1980) and aquatic (Mulkins-Phillips and Stewart 1974; Buckley et al. 1976; Ward and Brock 1976) ecosystems, the fraction of the total heterotrophic community represented by hydrocarbon-utilizing bacteria and fungi is highly variable, with reported frequencies ranging from

6 % (Jones et al. 1970) to 82 % (Pinholt et al. 1979) for soil fungi, 0.13 % (Jones et al. 1970) to 50 % (Pinholt et al. 1979) for soil bacteria, and 0.003 % (Hollaway et al. 1980) to 100 % (Mulkins-Phillips and Stewart 1974) for marine bacteria. Both bacteria and fungi are relatively plentiful in soil, and members of both groups contribute to the biodegradation of hydrocarbons (Bossert and Bartha 1984). Hydrocarbon-utilizing bacteria (Jensen 1975; Pinholt et al. 1979; Atlas et al. 1980) and fungi (Llanos and Kjoller 1976; Pinholt et al. 1979; Atlas et al. 1980) are readily isolated from soil and the application of oil or oily wastes to soil results in increased numbers of bacteria and fungi (Jensen 1975; Llanos and Kjoller 1976; Pinholt et al. 1979). In the only published comparative study of hydrocarbon degradation by bacteria and fungi in soil, Song et al. (1986) observed that 82 % of n-hexadecane mineralization in a sandy loam was attributed to bacteria and only 13 % was attributed to fungi. Individual organisms can metabolize only a limited range of hydrocarbon substrates (Britton 1984), so that assemblages of mixed populations with overall broad enzymatic capacities are required to degrade complex mixtures of hydrocarbons such as crude oil in soil (Bossert and Bartha 1984), freshwater (Cooney 1984), and marine (Atlas 1981; Floodgate 1984) environments. The ability to degrade and/or utilize hydrocarbon substrates is exhibited by a wide variety of bacterial and fungal genera. Floodgate (1984) lists 25 genera of hydrocarbon-degrading bacteria and 27 genera of hydrocarbon-degrading fungi which have been isolated from the marine environment; a similar compilation by Bossert and Bartha (1984) for soil isolates includes 22 genera of bacteria and 31 genera of fungi. Based on the number of published reports, the most important hydrocarbon-degrading bacteria in both marine and soil environments are *Achromobacter*, *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Flavobacterium*, *Nocardia* and *Pseudomonas* sp. and the coryneforms; the importance of hydrocarbon-degrading strains of the salt-requiring *Vibrio* sp. is limited to the marine environment. Austin et al. (1977), in a numerical taxonomy study of petroleum-degrading bacteria from Chesapeake Bay water and sediment, found that *Pseudomonas*, *Micrococcus*, and *Nocardia* sp., members of the family *Enterobacteriaceae*, *Actinomycetes*, and coryneforms made up 95 % of the isolates. Among the fungi, *Aureobasidium*, *Candida*, *Rhodotorula* and *Sporobolomyces* sp. are the most common marine isolates and *Trichoderma* and *Mortierella* sp. are the most common soil isolates. Hydrocarbon-degrading *Aspergillus* and *Penicillium* sp. have been frequently isolated from both environments. Based on the work of Kirk and Gordon (1988), the truly marine, beach-adapted genera *Corollospora*, *Dendryphiella*, *Lulworthia*, and *Varicosporina* should be added to the list. Fungal genera, namely *Amorphoteca*, *Neosartorya*, *Talaromyces*, and *Graphium*, and yeast genera, namely *Candida*, *Yarrowia*, and *Pichia*, were isolated from

petroleum-contaminated soil and proved to be potential organisms for hydrocarbon degradation (Chaillan et al. 2004). Singh (2006) also reported a group of terrestrial fungi, namely *Aspergillus*, *Cephalosporium*, and *Penicillium*, which were also found to be potential degraders of crude oil hydrocarbons. The yeast species, namely *Candida lipolytica*, *Rhodotorula mucilaginosa*, *Geotrichum* sp. and *Trichosporon mucoides*, when isolated from contaminated water were noted to degrade petroleum compounds (Bogusławska-Was and Dabrowski 2001). Walker and Colwell (1974) reported that Chesapeake Bay bacteria accounted for all of the utilization of model petroleum at 0 and 5 °C, while at 10 °C, yeasts but not filamentous fungi contributed to the utilization of the petroleum. A hydrocarbon-degrading fungus, *Cladosporium resinae*, was responsible for 20–40 % of the degradation of petroleum when added to the inoculum. Algae and protozoa are important members of the microbial community in both aquatic and terrestrial ecosystems, but the extent of their involvement in hydrocarbon biodegradation is largely unknown. Walker et al. (1975) isolated an alga, *Prototheca zopfii*, which was capable of utilizing crude oil and a mixed-hydrocarbon substrate and exhibited extensive degradation of n- and isoalkanes, as well as aromatic hydrocarbons. Cerniglia et al. (1980) observed that nine cyanobacteria, five green algae, one red alga, one brown alga, and two diatoms could oxidize naphthalene. Protozoa, by contrast, have not been shown to utilize hydrocarbons. Rogerson and Berger (1981) found no direct utilization of crude oil by protozoa cultured on hydrocarbon-utilizing yeasts and bacteria. Overall, the limited available evidence does not appear to suggest an ecologically significant role for algae and protozoa in the degradation of hydrocarbons in the environment (O'Brien and Dixon 1976; Bossert and Bartha 1984). Some hydrocarbon-degrading microorganisms are listed in Table 3.

Table 3 Some hydrocarbon-degrading microorganisms (Adapted from Desai and Pranav 2006)

Crude oil component	Microorganism
Saturates	<i>Arthrobacter</i> sp., <i>Acinetobacter</i> sp., <i>Candida</i> sp., <i>Pseudomonas</i> sp., <i>Rhodococcus</i> sp., <i>Streptomyces</i> sp., <i>Bacillus</i> sp., <i>Aspergillus japonicus</i>
Monocyclic aromatic hydrocarbons	<i>Pseudomonas</i> sp., <i>Bacillus</i> sp., <i>B. stercorophilus</i> , <i>Vibrio</i> sp., <i>Nocardia</i> sp., <i>Corynebacterium</i> sp., <i>Achromobacter</i> sp.
Polycyclic aromatic hydrocarbons	<i>Arthrobacter</i> sp., <i>Bacillus</i> sp., <i>Burkholderia cepacia</i> , <i>Pseudomonas</i> sp., <i>Mycobacterium</i> sp., <i>Xanthomonas</i> sp., <i>Phanerochaete chrysosporium</i> , <i>Anabaena</i> sp., <i>Alcaligenes</i>
Resins	<i>Pseudomonas</i> sp., Members of <i>Vibrionaceae</i> , <i>Enterobacteriaceae</i> , <i>Moraxella</i> sp.

Mechanism of petroleum hydrocarbon degradation

The most rapid and complete degradation of the majority of organic pollutants is brought about under aerobic conditions. The initial intracellular attack of organic pollutants is an oxidative process and the activation as well as incorporation of oxygen is the enzymatic key reaction catalyzed by oxygenases and peroxidases. Peripheral degradation pathways convert organic pollutants step by step into intermediates of the central intermediary metabolism; for example, the tricarboxylic acid cycle. Biosynthesis of cell biomass occurs from the central precursor metabolites, such as acetyl-CoA, succinate, and pyruvate. Sugars required for various biosyntheses and growth are synthesized by gluconeogenesis. The degradation of petroleum hydrocarbons can be mediated by specific enzyme systems. Other mechanisms involved are (1) attachment of microbial cells to the substrates and (2) production of biosurfactants (Hommel 1990). The uptake mechanism linked to the attachment of cells to oil droplets is still unknown but production of biosurfactants has been well studied. Biosurfactants can act as emulsifying agents by decreasing the surface tension and forming micelles. The microdroplets encapsulated in the hydrophobic microbial cell surface are taken inside and degraded. Biosurfactants produced by some microorganisms are listed in Table 5, below. Cameotra and Singh (2008) reported bioremediation of oil sludge using biosurfactants; the microbial consortium used in their study consisted of two isolates of *Pseudomonas aeruginosa* and one isolate of *Rhodococcus erythropolis* from oily sludge contaminated soil. Ninety percent of the hydrocarbons were degraded by the consortium after 6 weeks in liquid culture. The objective of their study was to test the ability of the consortium to degrade sludge hydrocarbons in two separate field trials and to assess the effect of two additives—a nutrient mixture and a crude biosurfactant preparation—on the efficiency of the process. The biosurfactant used was identified as a mixture of 11 rhamnolipid congeners and was produced by a consortium member. The observation of their study showed that 91 % of the hydrocarbon content from the soil contaminated with 1 % (v/v) crude oil sludge was degraded by the consortium after 5 weeks. About 91–95 % depletion of the hydrocarbon content was reported with the separate use of any one additive along with the consortium after 4 weeks. The crude biosurfactant preparation proved to be a more effective enhancer of degradation. When both the additives were combined with the consortium, more than 98 % hydrocarbon depletion was achieved. This observation suggested that hydrocarbon remediation could be successfully achieved through the use of a crude biosurfactant (Das and Chandran 2011). Biosurfactants produced by some microorganisms are listed in Table 5.

Role of enzymes in degradation of hydrocarbons

Cytochrome P450 alkane hydroxylases constitute a super family of ubiquitous heme-thiolate monooxygenases, which play an important role in the microbial degradation of oil, chlorinated hydrocarbons, fuel additives, and many other compounds (Van Beilen and Funhoff 2007). Depending on the chain length, enzyme systems are required to introduce oxygen in the substrate to initiate biodegradation. Higher eukaryotes generally contain several different P450 families that consist of large number of individual P450 forms that may contribute as an ensemble of isoforms to the metabolic conversion of a given substrate. In microorganisms such as P450, multiplicity can only be found in a few species (Zimmer et al. 1996). The cytochrome P450 enzyme system was found to be involved in the biodegradation of petroleum hydrocarbons (Das and Chandran 2011). The capability of several yeast species to use n-alkanes and other aliphatic hydrocarbons as a sole source of carbon and energy is mediated by the existence of multiple microsomal Cytochrome P450 forms. These cytochrome P450 enzymes had been isolated from yeast species such as *Candida maltosa*, *Candida tropicalis*, and *Candida apicola* (Scheuer et al. 1998). The diversity of alkane oxygenase systems in prokaryotes and eukaryotes that are actively participating in the degradation of alkanes under aerobic conditions, such as Cytochrome P450 enzymes, integral membrane di-iron alkane hydroxylases (e.g., *alkB*), soluble di-iron methane monooxygenases, and membrane-bound copper containing methane monooxygenases, have been discussed by Van Beilen and Funhoff (2005). Enzymes and Biosurfactants involved in the biodegradation of petroleum hydrocarbon are listed in Tables 4 and 5.

Genetic aspects of petroleum hydrocarbon degradation

Genetic factors play an important role in conferring biodegradation potentials on microorganisms. Plasmid DNA plays an important role in genetic adaptation, as it represents a highly mobile form of DNA which can be transferred via conjugation or transformation and can impart novel phenotypes, including the ability to transmit hydrocarbon-oxidation to a recipient organism. The pathways for the metabolism of naphthalene, salicylat, camphor, octane, xylene and toluene have been encoded on plasmids in *Pseudomonas* sp. (Chakrabarty 1976). The ability to degrade more recalcitrant components of petroleum, such as the aromatic fractions, is generally plasmid mediated (Cerniglia 1984b). The observed increase in the study of genetics paralleled the development of advances in molecular biology, particularly the application of recombinant DNA technology (Singer and Finnerty 1984b), gene probes (Barriault and Sylvestre 1993), and polymerase chain reaction (PCR) technology. Many bacterial catabolic pathways are specified by conjugative plasmids (Frantz and Chakrabarty 1986). These plasmids are readily transferred laterally into new host bacteria, thereby enhancing the metabolic potential of other members of an ecosystem (Okoh 2006).

Phytoremediation

Phytoremediation is an emerging technology that uses plants in the cleanup of environmental pollution, with hydrocarbons and other hazardous substances. Advantages of using phytoremediation include cost-effectiveness, aesthetic advantages,

Table 4 Enzymes involved in biodegradation of petroleum hydrocarbons

Microorganism	Enzyme	Substrate	Reference
<i>Methylococcus</i> <i>Methylosinus</i> <i>Methylocystis</i> <i>Methylomonas</i> <i>Methylocella</i> <i>Methylobacter</i>	Soluble Methane Monooxygenases	C1–C8 alkanes alkenes and cycloalkanes	McDonald et al. 2006
<i>Methylococcus</i> , <i>Methylocystis</i> <i>Pseudomonas</i> <i>Burkholderia</i> <i>Rhodococcus</i> , <i>Mycobacterium</i>	Particulate Methane Monooxygenases AlkB related Alkane Hydroxylases	C1–C5 (halogenated) alkanes and cycloalkanes C5–C16 alkanes, fatty acids, alkyl benzenes, cycloalkanes and so forth	McDonald et al. 2006 Jan et al. 2003
<i>Candida maltosa</i> <i>Candida tropicalis</i> <i>Yarrowia lipolytica</i>	Eukaryotic P450	C10–C16 alkanes, fatty acids	Iida et al. 2000
<i>Acinetobacter</i> <i>Caulobacter</i> <i>Mycobacterium</i> <i>Acinetobacter</i>	Bacterial P450 oxygenase system Dioxygenases	C5–C16 alkanes, cycloalkanes C10–C30 alkanes	Van Beilen et al. 2006 Maeng et al. 1996

Table 5 Biosurfactants produced by some microorganisms

Microorganism	Biosurfactant	Reference
<i>Candida bombicola</i>	Sophorolipids	Daverey and Pakshirajan 2009
<i>Pseudomonas aeruginosa</i>	Rhamnolipids	Kumar et al. 2008
<i>Candida tropicalis</i>	Lipomannan	Muthuswamy et al. 2008
<i>Pseudomonas fluorescens</i>	Rhamnolipids	Mahmound et al. 2008
<i>Bacillus subtilis</i>	Surfactin	Youssef et al. 2007
<i>Aeromonas</i> sp.	Glycolipid	Ilori et al. 2005
<i>Bacillus</i> sp.	Glycolipid	Tabatabaee et al. 2005

and long-term applicability. The Alabama Department of Environmental Management studied a 1,500-cubic-yard soil site, of which 70 % of the baseline samples contained over 100 ppm of total petroleum hydrocarbon (TPH). After 1 year of vegetative cover, approximately 83 % of the samples were found to contain less than 10-ppm TPH. Removal of total petroleum hydrocarbon (TPH) at several field sites contaminated with crude oil, diesel fuel, or petroleum refinery wastes at initial TPH concentrations of 1,700–16,000 mg/kg were also investigated (Hecht and Badiane 1998; Nedunuri et al. 2000). Plant growth was found to vary depending upon the species. The presence of some species led to greater TPH disappearance than with other species or compared to unvegetated soil. Among tropical plants tested for use in the Pacific Islands, three coastal trees, kou (*Cordia subcordata*), milo (*Thespesia populnea*), and kiawe (*Prosopis pallida*), and the native shrub beach naupaka (*Scaevola serica*) were found to tolerate field conditions and facilitated the cleanup of soils contaminated with diesel fuel (U.S. Army Corps of Engineers 2003). Microbial degradation in the rhizosphere might be the most significant mechanism for removal of diesel range organics in vegetated contaminated soils (Miya and Firestone 2001). This occurs because contaminants such as PAHs are highly hydrophobic, and their sorption to soil decreases their bioavailability for plant uptake and phytotransformation (Das and Chandran 2011).

Future prospects

The bioremediation studies after the Exxon Valdez oil spill demonstrated the promise of this technology for cleaning up a petroleum-contaminated site (NOAA Technical Memorandum 1997). Bioremediation was more extensive in 1990, with 378 of the 587 shoreline segments treated that year receiving bioremediation application (Chianelli et al. 1991). To determine the extent of the natural removal of oil, the National Oceanic and Atmospheric Administration (NOAA) conducted monitoring programs which determined that removal of surface oil from exposed shorelines was about 90%; for sheltered shorelines and those with intermittent energy, the removal rates were 70%. For subsurface oil, the removal rate was

approximately 55%, though this varied with the depth of the subsurface oil impacts (Michel et al. 1991). It has been estimated that a beach damaged by an oil spill could be returned to its natural condition in as little as 2–5 years with bioremediation treatment; otherwise it would take 10 years or more to reach this condition. Ten years after the Exxon Valdez accident, oil residues still remain below the surface in the spill-affected bays where contamination problems persist and negatively impact fishery activities. This fact clearly indicates the necessity for further development of bioremediation technologies to manage marine and terrestrial oil pollution. The microorganisms which play major roles in bioremediation, and their nutrient requirements (carbon, nitrogen, phosphorous, etc.) and environmental requirements (oxygen or an alternative electron acceptor, temperature, redox potential, salinity, pH, etc.) should be further researched and confirmed. Such knowledge would allow the manipulation of environmental factors that may limit or prevent the biodegradation of petroleum in environment. Undertaking bioremediation cannot be done without the agreement of local communities. Their concerns about bioremediation, a relatively new technology, should be mitigated by scientists who can explain to the local people the results of hydrocarbon contamination tests and microorganism tests, especially in regard to risk assessment. The identification of the Alcanivorax group as a major population that arose during a bioremediation treatment would be useful information to help convince local residents that the propagation of harmful microorganisms would not occur during bioremediation (Harayama et al. 1999).

Conclusion

Bioremediation is a very popular and promising technology for the remediation of environments contaminated with petroleum hydrocarbon. Petroleum hydrocarbon contamination causes various hazardous effects on ecosystems and humans. Physical and chemical methods used for the treatment are not that much more efficient and are very costly compared to bioremediation, which is less expensive and more efficient. Various physical and chemical factors, such as temperature, oxygen, pH, nutrients etc. and the type of

contaminants, metabolic capabilities of the indigenous microbial population, and type of plant species used in phytoremediation affect the degradation rate of petroleum hydrocarbon. Microbial degradation of petroleum hydrocarbon is accomplished by a diversity of microorganisms because they have enzyme systems to degrade and utilize different hydrocarbons as a source of carbon and energy. Bacteria are the most powerful degrading agent compared to fungi, algae and protozoa. Genetically engineered microorganisms (GEMs) and phytoremediation also play an important role in the remediation of petroleum hydrocarbon from environment. Several GEMs have been successfully constructed and experimentally proven to have a higher degradative capability and utility for bioremediation under laboratory conditions (Furukawa 2003; Pieper and Reineke 2000; Lovley 2003). In spite of such studies, applying GEMs in situ has been limited by the risks associated with horizontal gene transfer and uncontrolled proliferation of the introduced GEMs (Velkov 2001; Boopathy 2000). Austria, France, Greece, Hungary, Germany and Luxembourg have all banned the cultivation of genetically modified organisms (GMOs). Angola has banned imports of all GMO produce, except for food aid provided it was milled; Zambia banned import of all GMOs as well. Therefore, from this present review we conclude that the microbial degradation of petroleum hydrocarbons can be considered as a key component in the cleanup strategy for petroleum hydrocarbon remediation.

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