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MINI-REVIEW

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Biodegradation and bioremediation of hydrocarbons in extreme environments

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Abstract Many hydrocarbon-contaminated environments are characterized by low or elevated temperatures, acidic or alkaline pH, high salt concentrations, or high pressure. Hydrocarbon-degrading microorganisms, adapted to grow and thrive in these environments, play an important role in the biological treatment of polluted extreme habitats. The biodegradation (transformation or mineralization) of a wide range of hydrocarbons, including aliphatic, aromatic, halogenated and nitrated compounds, has been shown to occur in various extreme habitats. The biodegradation of many components of petroleum hydrocarbons has been reported in a variety of terrestrial and marine cold ecosystems. Cold-adapted hydrocarbon degraders are also useful for wastewater treatment. The use of thermophiles for biodegradation of hydrocarbons with low water solubility is of interest, as solubility and thus bioavailability, are enhanced at elevated temperatures. Thermophiles, predominantly bacilli, possess a substantial potential for the degradation of environmental pollutants, including all major classes. Indigenous thermophilic hydrocarbon degraders are of special significance for the bioremediation of oil-polluted desert soil. Some studies have investigated composting as a bioremediation process. Hydrocarbon biodegradation in the presence of high salt concentrations is of interest for the bioremediation of oil-polluted salt marshes and industrial wastewaters, contaminated with aromatic hydrocarbons or with chlorinated hydrocarbons. Our knowledge of the biodegradation potential of acidophilic, alkaliphilic, or barophilic microorganisms is limited.

Introduction

Biodegradation is the metabolic ability of microorganisms to transform or mineralize organic contaminants into less harmful, non-hazardous substances, which are then integrated into natural biogeochemical cycles. The intensity of biodegradation is influenced by several factors, such as nutrients, oxygen, pH value, composition, concentration and bioavailability of the contaminants, chemical and physical characteristics and the pollution history of the contaminated environment. Bioremediation, a non-destructive, cost- and treatment-effective and sometimes logistically favorable cleanup technology, attempts to accelerate the naturally occurring biodegradation of contaminants through the optimization of limiting conditions (Norris 1994; Allard and Neilson 1997; Alexander 1999).

Many environments are characterized by low or elevated temperatures, acidic or alkaline pH, high salt concentrations, or high pressure. Extremophilic microorganisms are adapted to grow and thrive under these adverse conditions. Hydrocarbon degrading extremophiles are thus ideal candidates for the biological treatment of polluted extreme habitats. In this review we summarize the recent developments, obtained both in laboratory and field studies, in biodegradation and bioremediation of hydrocarbon contaminants that are of environmental concern in extreme habitats.

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Low and high temperature environments

Temperature plays a significant role in controlling the nature and the extent of microbial hydrocarbon metabolism, which is of special significance for in situ bioremediation. Bioavailability and solubility of less soluble hydrophobic substances, such as aliphatic and polyaromatic hydrocarbons, are temperature dependent. A temperature increase (decrease) effects a decrease (increase) in viscosity, thereby affecting the degree of distribution, and an increase (decrease) in diffusion rates of organic compounds. Therefore, higher reaction rates due to smaller boundary layers are expected at elevated temperatures. The increased (decreased) volatilization and solubility of some hydrocarbons at elevated (low) temperature affects

toxicity and allows biotransformations with high (low) substrate concentrations (Müller et al. 1998; Whyte et al. 1998; Niehaus et al. 1999).

Cold-adapted microorganisms

Cold-adapted, psychrophilic and psychrotrophic microorganisms are able to grow at temperatures around 0°C. They are widely distributed in nature since a large part of the Earth's biosphere is at temperatures below 5°C (Margesin and Schinner 1999a). Psychrophiles have an optimum growth temperature of ≤15°C and do not grow above 20°C, whereas psychrotrophs (cold-tolerant) have optimum and maximum growth temperatures above 15°C and 20°C, respectively (Morita 1975). Cold-adapted indigenous microorganisms play a significant role in the in situ biodegradation of hydrocarbons in cold environments, where ambient temperatures often coincide with their growth temperature range.

Petroleum hydrocarbons

Petroleum hydrocarbons belong to the most widespread contaminants of water and soil. The biodegradation of many components of petroleum hydrocarbons has been reported in a variety of terrestrial and marine cold ecosystems, including Arctic (Braddock et al. 1997), alpine (Margesin 2000) and Antarctic soils (Aislabie et al. 1998), Arctic seawater (Siron et al. 1995) and Antarctic seawater and sediments (Delille et al. 1998; Delille and Delille 2000). Comparable conclusions resulted from these studies (reviewed by Margesin and Schinner 1999b). Cold habitats possess sufficient indigenous microorganisms, psychrotrophic bacteria being predominant. They adapt rapidly to the contamination, as demonstrated by significantly increased numbers of oil degraders shortly after a pollution event. Significant numbers of cold-adapted hydrocarbon degraders were even found in contaminated soils from the most northerly inhabited station in the world, Canadian Forst Station-Alert (Whyte et al. 1999). The winter period was found to be critical for an oil spill in Arctic/sub-Arctic environments because of the reduced biodegradation under icy conditions; the temperature threshold for significant oil biodegradation is around 0°C (Siron et al. 1995).

Bioaugmentation of contaminated cold sites with hydrocarbon degraders has been tested as a bioremediation strategy. Most studies showed that the indigenous microbial populations degrade hydrocarbons more efficiently than the introduced strains. However, bioaugmentation may result in a shorter hydrocarbon acclimation period. Inoculation of contaminated Arctic soils with consortia (Whyte et al. 1999) or with alkane-degrading *Rhodococcus* sp. (Whyte et al. 1998) decreased the lag time and increased the rate of C_{16} mineralization at 5°C. However, NPK fertilization alone had a comparable effect on hydrocarbon loss like fertilization plus bioaugmentation, this has been shown both in chronically oil-polluted Arctic soil (Whyte et al. 1999) and in artificially diesel oil-contaminated alpine soils (Margesin and Schinner 1997a, b).

Several oil tanker accidents, and those of the Bahia Paraiso in Antarctica and of the Exxon Valdez in Alaska during 1989, demonstrated that the effectiveness of oil biodegradation in cold environments was limited by the availability of nutrients such as N and P, confirming results obtained in laboratory and field studies (for a review see Margesin and Schinner 1999b). The fate of oil from the Alaskan spill (estimated 35,500 m^3 of crude oil) has been determined from the most complete and accurate mass balance of any oil spill. The most significant term in the mass balance was about 50% of the oil that biodegraded either in situ on beaches or in the water column (Wolfe et al. 1994). Shortly after the spill, elevated hydrocarbon mineralization (hexadecane, naphthalene, phenanthrene) potentials and significantly (about 10^4-fold) increased numbers of oil degraders (10^6 cfu g^{-1} oiled beach material) were found (Braddock et al. 1996).

Nutrients may influence biodegradation of various oil components to a different extent. Biodegradation of aromatic hydrocarbons appears to be sensitive to pH and possibly also to by-products of the biodegradation of the saturate fraction, which could explain the persistence of aromatic petroleum hydrocarbons in the environment. Foght et al. (1999) investigated the role of the N source in biodegradation of crude oil components by a defined bacterial consortium, consisting of three degraders of aromatic hydrocarbons and heterocycles and three degraders of saturated hydrocarbons, under cold, marine conditions (10°C). Nitrate did not affect the pH, whereas ammonium amendment led to progressive acidification, accompanied by an inhibition of the degradation of aromatic, particularly polycylic aromatic hydrocarbons (PAHs). These compounds were degraded or co-metabolized in the absence of nutrients where the pH remained almost unchanged. The best overall biodegradation was observed in the presence of nitrate without ammonium, plus high phosphate buffering. However, as a disadvantage of nitrate, significant emulsification of the oil was observed.

Besides the nutrients, the physical environment is also important for hydrocarbon biodegradation. This has been demonstrated in sub-Antarctic intertidal beaches (Delille and Delille 2000) and in Arctic soils (Mohn and Stewart 2000). Soil N and concentrations of total petroleum hydrocarbons together accounted for 73% of the variability of the lag time for dodecane mineralization at 7°C in Arctic soils. High total carbon concentrations were associated with high mineralization rates; high sand content resulted in longer half-times for mineralization. Dodecane mineralization was limited by both N and P; mineralization kinetics varied greatly among different soils (Mohn and Stewart 2000).

The microbial catabolic pathways responsible for the degradation of petroleum hydrocarbons, including the *alk* (C_5 to C_{12} *n*-alkanes), *xyl* (toluene; aromatic hydro-

carbons) and *nah* (naphthalene; PAHs) pathways, are widespread in cold regions (Sotsky and Atlas 1994; Whyte et al. 1997, 1999). So far, only *alkB*$^+$ psychrotrophs but no *alkB*$^+$ psychrophiles have been described from a variety of Arctic samples (Whyte et al. 1999). Rarely microorganisms possess both the *alk* and *nah* catabolic pathways. Whyte et al. (1997) have demonstrated that both pathways, located on separate plasmids, can naturally coexist in the same bacterium. Two hydrocarbon-degrading psychrotrophic *Pseudomonas* spp. strains degraded *n*-alkanes from C_5 to C_{12}, toluene and naphthalene both at 5°C and at 25°C.

Alkanes

Alkanes are major constituents of petroleum hydrocarbons. Cold-tolerant bacteria, isolated from oil-contaminated soils in Antarctica, were able to degrade *n*-alkanes (C_6 to C_{20}) typical of the hydrocarbon contaminants that persist in Antarctic soil (Bej et al. 2000). Representative isolates were identified as *Rhodococcus* species, they retained metabolic activity at sub-zero temperatures of –2°C. A psychrotrophic *Rhodococcus* sp. from Arctic soil (Whyte et al. 1998) utilized a broad range of aliphatics (C_{10} to C_{21} alkanes, branched alkanes, and a substituted cyclohexane) present in diesel oil at 5°C. The strain mineralized the short-chain alkanes (C_{10} and C_{16}) to a significantly greater extent (by a factor of about 2–3) than the long-chain alkanes (C_{28} and C_{32}) at 0 and 5 °C. The decreased bioavailability of the long-chain alkanes at low temperature (many form crystals at 0 °C) may be responsible for their increased recalcitrance, which affects in situ bioremediation in cold climates. The application of cold-active solubilizing agents could be useful for enhancing hydrocarbon bioavailability. Two hydrocarbon-degrading Antarctic marine bacteria, identified as *Rhodococcus fascians*, produced bioemulsifiers when grown with *n*-alkanes as the sole carbon source. The strains utilized hexadecane and biphenyl as sole carbon sources at temperatures ranging from 4 to 35°C, the optimum temperature was 15–20°C (Yakimov et al. 1999). Psychrophilic strains with high oil-oxidizing and bioemulsifying activities were also described by Chugunov et al. (2000).

Aromatic hydrocarbons

Benzene, toluene, ethylbenzene, xylenes (BTEX). Underground fuel leaks contribute significantly to the contamination of groundwater. Non-oxygenated monoaromatic hydrocarbons, such as BTEX, are of particular concern. The high water solubility of BTEX enables them to migrate in the subsurface and contaminate drinking water. Bradley and Chapelle (1995) demonstrated the technical feasibility of in situ bioremediation of hydrocarbons in cold groundwater systems. Rapid aerobic toluene mineralization was demonstrated in sediments from a cold (mean groundwater temperature 5°C) petroleum-contaminated aquifer in Alaska. The mineralization rate obtained at 5°C in this aquifer was comparable to that measured in sediments from a temperate aquifer (mean temperature 20°C). Rates of overall microbial metabolism in the two sediments were comparable at their respective in situ temperatures. Thus, hydrocarbon biodegradation rates in cold groundwater systems are not necessarily lower than in temperate systems. This study demonstrated that activity measurements should be performed at the prevailing in situ temperature in order to obtain a realistic estimate of the naturally occurring biodegradation.

Intrinsic hydrocarbon bioremediation was also demonstrated in a shallow aquifer underlying a natural gas production (Gieg et al. 1999). Changes were monitored over 4 years. All BTEX compounds were biodegraded under sulfate-reducing conditions, toluene was also degraded under methanogenic conditions. Other authors (Weiner and Lovley 1998) noted the persistence of benzene in the sulfate reduction zones of petroleum-contaminated aquifers. This benzene persistence was attributed to the lack of benzene-oxidizing sulfate reducers in the aquifer sediments, rather than to the inability of such microorganisms to grow and metabolize under freshwater conditions. In order to obtain accelerated benzene bioremediation in contaminated anaerobic aquifers, Weiner and Lovley (1998) proposed to supplement aquifer sediments with benzene-oxidizing sulfate reducers. Such a treatment could be an efficient alternative to commonly employed aerobic strategies.

Nearly 20 years after the NARL oil spill in Barrow, Alaska, benzene mineralization potentials at 10°C (0.1–0.5 mg benzene mineralized l^{-1} day^{-1}) were comparable to those of temperate systems. Mineralization in both groundwater and soil was higher in samples from contaminated sites than from the reference site. Nevertheless, microbial processes and contaminant transport were limited by the short annual thaw season (Braddock and McCarthy 1996). We investigated biodegradation of BTEX compounds in soils (R. Margesin and F. Schinner, unpublished data). Within 18 days, a BTEX loss of only 25% of the initial contamination (400 mg total BTEX kg^{-1} soil) was obtained in a high-alpine soil at 10°C, which was significantly lower than BTEX loss (76%) measured in soil from a temperate area at 20°C. NPK fertilization enhanced benzene and ethylbenzene biodegradation in particular.

Phenol. Phenolic compounds are common constituents of wastewaters from the oil industry. The bacteria involved in biodegradation may strongly influence the temperature-dependent performance of wastewater treatment processes. Onysko et al. (2000) determined the effect of temperature (10–25°C), appropriate for wastewater treatment, on growth and phenol degradation kinetics of psychrotrophic *Pseudomonas putida*. According to the substrate-inhibition model used, based on the Haldane equation and valid for substrate concentrations less than 400 mg l^{-1}, the maximum specific growth rate (μ_{max}) and the Monod constant (K_s) increased with increasing tem-

perature. The simultaneous increase of the inhibition constant (K_i) indicated an increased degree of inhibition at low temperatures.

Another psychrotrophic *Pseudomonas putida* was reported to remove a wide variety of phenolic compounds from wastewater under aerobic and pH-neutral conditions at temperatures ranging from 1 to 35°C (Pillis and Davis 1985). This strain can be used in trickling filter systems, activated sludge treatments, and outdoor lagoons, either alone or in combination with other microorganisms conventionally used in waste treatment. It can be cultured in wastewater using either a batch process, a semi-continuous or a continuous process, for a sufficient amount of time (24 h to 4 weeks, depending on the temperature, the volume to be treated, and the concentration of the contamination) to achieve a significant reduction. The resulting water is suitable for discharge into rivers and streams after conventional processing. Lagoon efficiency is often low in winter when microorganisms are less active. The use of this strain allowed a reduction in the amount of steam required in winter for lagoon heating in order to achieve normal lagoon operation, with a considerable saving in energy costs.

Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are frequent soil contaminants. Little is known about biodegradation of PAHs in cold habitats. Cold-tolerant isolates from oil-polluted Antarctic soils utilized naphthalene, phenanthrene and fluorene, as well as BTEX, as the sole carbon and energy source (Aislabie et al. 2000). Representative strains were identified as *Sphingomonas* or *Pseudomonas* spp. Mineralization of naphthalene in polluted soil, determined at 8°C, was 4.02% and 1.42% CO_2 day^{-1} with and without N fertilization, respectively, and was considerably higher than mineralization of hexadecane (Aislabie et al. 1998). In a field study on the biodegradation of dispersed crude oil in cold and icy seawater (–1.8 to 5.5°C), half-life times of PAHs ranged from 1.5–1.7 days (naphthalene) to 2.4–7.5 days (phenanthrene) under favorable conditions, i.e. at a temperature above 0°C and with effective chemical dispersion (Siron et al. 1995).

Chlorinated hydrocarbons

Industrial zones involved in pulp and paper manufacturing and precision machining are contaminated to a large extent by chlorinated hydrocarbons such as dichlororethylene (DCE) and trichloroethylene (TCE). Mihara et al. (1999) described a novel gram-negative, rod-shaped microbial strain, which is able to degrade DCE and TCE efficiently, as well as aromatic hydrocarbons (phenol, toluene, all three isomers of cresol) at temperatures as low as 4°C. The strain does not require an inducer such as phenol, toluene or cresol for degrading volatile organic compounds (VOCs). No growth occurs under extremely acidic or alkaline conditions or at high salt concentrations. The strain is used in a patented low-temperature bioremediation process to treat contaminated wastewater, soil and/or air (i.e. the surrounding gaseous phase of the polluted environment) in an open or closed system. It can be introduced directly into an aqueous medium or into soil (sprayed onto the soil surface, or introduced into soil underground).

Aquifers are frequently contaminated with TCE. Its behavior as a dense non-aqueous-phase liquid makes removal by classical pump-and-treat methods unsatisfactory. Moran and Hickey (1997) showed that in situ bioremediation of TCE-contaminated groundwater is feasible at 12°C, a temperature typical of shallow aquifers. Biostimulation of ammonia oxidizers with ammonia was effective at the low temperature, whereas biostimulation of methanotrophs with methane was not successful. Thus, the stimulation of ammonia oxidizers could be an alternative for remediating TCE-contaminated aquifers. Ammonium is inexpensive and non-toxic and has a higher aqueous solubility than methane. However, a problem arises from the production of nitrate from ammonium.

The extensive use of chlorinated phenols and their derivatives (e.g. as wood preservatives) in agriculture and industry has resulted in extensive environmental contamination. High-rate chlorophenol (tri-, tetra- and pentachlorophenol) biodegradation in groundwater was achieved in an aerobic fluidized bed system at 5–7°C, and over 99.9% mineralization was obtained at a loading rate of 740 mg l^{-1} day^{-1}. The effluent quality was close to drinking water standard. This system has the advantage that it can be operated and maintained at actual groundwater temperatures and avoids the heating costs (an increase of the process temperature from 7 to 25°C increases annual operating costs by a factor of 2.5; Järvinen et al. 1994).

Aerobic degradation of polychlorinated biphenyls (PCBs) with up to three chlorine constituents has been shown to occur at 4°C in river sediment spiked with Aroclor 1242 (70 ppm on a dry weight basis). Loss of di- and trichlorobiphenyls was first indicated after 1.4 months, and >50% loss occurred within 5 months at 4°C (Williams and May 1997). Biphenyl mineralization by Arctic soil microorganisms was higher in contaminated than in uncontaminated soil slurries; typical mineralization rates at 7°C were 0.5–1 mg biphenyl g^{-1} dry soil day^{-1} (initial concentration 5–50 mg biphenyl l^{-1}; Mohn et al. 1997). The extent of PCB degradation (Aroclor 1221; initial concentration 50 mg l^{-1}) at the low temperature was significantly lower in soil slurries (14–40% removal) than in pure cultures (54–60% removal). Such isolates are not effective for bioremediation of PCB mixtures, but could be useful in a sequential process in which PCBs are first reductively dechlorinated by aerobic microorganisms. A comparison of the effect of temperature on PCB removal by mesophilic and cold-adapted degraders demonstrated that the PCB-degrading enzyme system of the Arctic bacteria is cold-adapted (Master and Mohn 1998).

Thermophilic microorganisms

Microorganisms that grow optimally above 40°C are designated as thermophiles. Most thermophiles known are moderate and show an upper temperature border of growth between 50 and 70°C. Optimal growth of extreme thermophiles and hyperthermophiles occurs at 70–80°C and above 80°C, respectively (Stetter 1998). Thermophiles, predominantly bacilli, possess a substantial potential for the conversion of environmental pollutants, including all major classes (Müller et al. 1998).

Petroleum hydrocarbons

Bioremediation has been evaluated in several studies as an option to treat the oil pollution remaining after the Gulf war in 1991. As a consequence of one of the most massive oil spills (estimated 0.95–1.27 Mm^3), about 770 km of the western coast of the Arabian Gulf and about 50 km^2 of the Kuwaiti desert were contaminated with crude oil from over 700 damaged oil wells. Most of the crude oil has been pumped out of the oil lakes, but the bottom sand remained heavily polluted to depths of 30 cm or more (Al-Daher et al. 1998). The Arabian Gulf is situated in a semi-arid region, characterized by high evaporation rates (200 cm $year^{-1}$) and high salinities (4–7%) (Khan and Al-Ajmi 1998). In the long hot summers of the region, the atmospheric temperature frequently exceeds 50°C, and the surface-soil temperature is considerably higher (Sorkhoh et al. 1993). The desert soil contains accumulated salt in the surface layer. Its extremely high salinity is attributed partly to the large volume of seawater used to extinguish the oil well fires, and the evaporation of this water (Balba et al. 1998). Such an environment is expected to be an ideal habitat for extremophilic microorganisms. As the first sign of self-cleaning indigenous activity, blue-green cyanobacterial mats, associated with oil, appeared over oil layers about 1 year after the pollution event. These mats have not been found to be associated with oil spills before, and appeared to be unique to the extreme environment of the Gulf (Sorkhoh et al. 1992). Sorkhoh et al. (1993) investigated 38 desert samples, taken from oil-contaminated areas in Kuwait. Thermophilic crude-oil utilizing bacteria occurred at a density of 3×10^3 to 1×10^7 g^{-1} soil. No thermophilic oil-utilizing fungi were found, and water and sediment samples from the Arabian Gulf were also free of thermophilic oil utilizers. All 368 isolates belonged to the genus *Bacillus*, the species *B. stearothermophilus* being predominant. Two strains degraded about 80–89% of the crude oil (5 g l^{-1}) within 5 days at their optimum growth temperature of 60°C, biodegradation was 3- to 4-fold lower at 40°C. The range of efficiently utilized hydrocarbons was narrow and included C_{15} to C_{17} *n*-alkanes. Growth of both strains with *n*-alkanes from C_6 to C_{14} and $>C_{17}$, as well as with several *n*-alkanes and aromatic hydrocarbons, was weak, if any. Al-Maghrabi et al. (1999) isolated two thermophilic *Bacillus* strains that degrade crude oil (20% v/v) at 40–45°C (the typical ambient temperature of the Persian Gulf region), one strain could grow with crude oil even at 80°C. The isolates survived under saline conditions typical of seawater and reservoir connate water. Optimal growth in the presence of 10% v/v crude oil and 2% salinity was observed at 60–80°C.

The efficiency of various bioremediation approaches to treat oil-polluted desert soil has been evaluated in several field studies. They all demonstrated the significance of indigenous oil-degrading bacteria, while inoculation with biodegraders was not effective (Radwan et al. 1995). Unfortunately, in these studies there is no exact indication of field temperatures. Due to the essential role of moisture in bioremediation, repeated irrigation of polluted desert soils is necessary. The use of seawater is unsuitable because of its inhibitory effect on hydrocarbon biodegradation (Radwan et al. 2000). Saltwater accelerates the soil water evaporation, as a consequence salt remains and accumulates at the soil surface. Only very diluted seawater (0.5% w/v NaCl) can be used to stimulate oil biodegradation (Radwan et al. 1995). The use of large volumes of freshwater in bioremediation of desert soils leads to the leaching of salts and the reduction of soil salinity. A decreased salt concentration was measured after land farming and windrow composting with continuous irrigation, while the salt content increased in non-irrigated controls due to water evaporation (Balba et al. 1998).

Radwan et al. (1998) proposed vegetation as a feasible approach for bioremediation of oil-polluted desert soil. The rhizosphere of the investigated wild desert plants and crop plants (*Vicia faba*, *Lupinus albus*) contained more hydrocarbon-utilizing bacteria than the soil alone. This effect was much more pronounced for plants growing in oil-polluted than in uncontaminated soil.

Balba et al. (1998) compared the efficiency of three bioremediation techniques in an oil lake area. In a 1-year field study, soil amended with N, P, compost and wood chips was subjected to land farming, windrow composting and bioventing. At the beginning of the study, the soil scraped from the surface layer contained 133–694 g total petroleum hydrocarbons kg^{-1} soil and was extremely salty (about 30–40% w/w NaCl). Both contamination and salinity gradually reduced with soil depth, and reached levels of 0.2–0.7 g hydrocarbons kg^{-1} soil and 0.03–0.3% w/w NaCl. After 12 months, a significant reduction in oil concentration was detected in all treatments. The loss of total alkanes was 91% (land farming) and 82% (the other two treatments), compared to 16–26% loss in untreated controls. The content of total petroleum hydrocarbons was reduced by 64% (static bioventing piles), 74% (windrow piles) and 83% (land farming). Despite the lower bioremediation efficiency of bioventing piles, this technique requires considerably lower operation and maintenance costs than other techniques; it also requires a much smaller site area, and less water for irrigation. Al-Daher et al. (1998) performed composting of oil-polluted (33–55 g kg^{-1} soil), NPK-fertilized desert soil in an oilfield. The applied windrow

soil pile system, subjected to regular irrigation and turnover, resulted in a reduction of up to 60% of the contamination within 8 months. The concentration of PAHs was reduced from 11–16 to 5–6 mg kg^{-1} soil, but PAHs with five or more rings resisted biodegradation. The soil (1 g) contained about 10^4 thermotolerant and 10^5 to 10^6 mesophilic hydrocarbon-utilizing bacteria.

Several studies investigated composting as a bioremediation process. Contaminated soil can be mixed with traditional composting substrates such as plant debris and sewage sludge. This mixture may be treated in a windrow or in an enclosed reactor. Composting offers the advantages of enhanced opportunities for co-metabolism and greater contaminant availability due to higher solubility and mass transfer at elevated temperatures. However, VOCs released during composting require additional cost-increasing treatment (Williams et al. 1992). The amount of organic amendment, the C:N ratio and the temperature profile are key factors affecting composting of soil contaminated with weathered hydrocarbons (17 g mineral oil and grease kg^{-1} soil) (Beaudin et al. 1999). A temperature of 50°C maintained for 29 days resulted in 68% hydrocarbon loss. The length of the thermophilic phase (50°C) correlated with the measured hydrocarbon degradation. The authors gave no information about the thermophilic microorganisms involved in composting. In another study, the maintenance of the thermophilic temperature was shown to be influenced by the contaminant concentration (Chung et al. 2000). During the composting of kerosene-contaminated soil, the temperature decreased significantly in the presence of 20% kerosene, but was maintained if the kerosene concentration was 5% or lower. After 64 days of composting, most of the hexadecane was volatilized, and only a small part was mineralized to CO_2. Decreasing the aeration rate reduced volatilization significantly and improved hexadecane biodegradation.

Aromatic hydrocarbons

Lugowski et al. (1997) developed and patented a process for the detoxification of liquid effluent streams. A mixture of thermophilic aerobic bacteria, comprising predominantly *Pseudomonas* species, is used to degrade a wide range of aromatic hydrocarbons, such as phenols, toluene, aniline, benzothiazole, lindane (hexachlorocyclohexane), and combinations thereof. For seeding, a 10–30 l quantity of the bacterial culture is grown at 42°C and stabilized at a density of 10^8 cells ml^{-1}. Bioreactor contents are maintained at a constant temperature of 40–42°C. The detoxified effluent streams can be further treated by conventional (i.e. municipal) sewage treatment facilities. An advantageous by-product of the process is heat energy that could be recycled.

BTEX. Chen and Taylor (1995, 1997a) isolated two thermophilic aerobic bacteria (*Thermus aquaticus* and *Thermus* sp.) that degrade BTEX co-metabolically. Resting cell suspensions of the strains degraded 10–40% of low BTEX concentrations (initial total concentrations were 2 and 7 mg l^{-1}) within 45 days at 60°C and 70°C. Only small fractions of benzene and toluene were metabolized to CO_2. Biodegradation was inhibited by higher BTEX concentrations, but was enhanced if strains were pregrown on catechol and *o*-cresol (Chen and Taylor 1995). Biomass formation (2.5 g cell dry weight l^{-1}) and BTEX degradation (specific activity of 2–10 nmol degraded BTEX mg^{-1} cell dw min^{-1}) by *Thermus* sp. were higher in fed-batch culture than in batch culture (Chen and Taylor 1997a). A slow to moderate specific growth rate of 0.02–0.07 h^{-1} favored BTEX degradation, while a high growth rate, such as 0.16 h^{-1}, was inhibiting. Biodegradation occurred over a broad temperature range (45–77°C).

Two anaerobic consortia, consisting of unidentified bacterial cocci, could grow on all BTEX compounds as sole carbon and energy sources at 45–75°C, 50°C being the optimal temperature (Chen and Taylor 1997b); 22–40% of an initial concentration of 117 mg total BTEX l^{-1} was degraded after 14 days at 50°C. Only a small fraction of toluene was mineralized to CO_2. Biodegradation was coupled by both consortia to sulfate reduction as well as to H_2S generation. No growth or BTEX metabolism occurred when sulfate was omitted. Thus, sulfate-reducing bacteria are most likely the principal species that carry out the biodegradation, while other thermophilic species may use the early water-soluble BTEX metabolites.

The above-described aerobic and anaerobic thermophiles are used for a patented in situ thermally enhanced remediation strategy (Taylor et al. 1998). The authors propose the coupling of bioremediation to in situ dynamic underground stripping (DUS), a primary decontamination effort for the rapid treatment of contaminated soil at significant depths. A targeted subsurface volume is heated (by steam injection and electrical resistance) to vaporize the trapped contaminants, these are then removed by vacuum extraction. However, even VOCs such as BTEX are not completely removed during vaporization. Due to the use of heat, the entire underground environment remains at elevated temperatures of 50–70°C for at least 60 days after the heat treatment. This enables thermophilic bacteria to metabolize the residual contaminants. Using the existing injection-withdrawal wells, the pregrown biodegraders are inoculated to enhance the removal of low levels of residual BTEX from fuel leaks, but also to treat chlorinated hydrocarbons, such as TCE and chloroform, from past solvent cleaning practices. The bioremediation process is terminated by lowering the temperature below 40°C. Such an in situ follow-up treatment could also be applied to fuel-contaminated plumes subjected to thermally enhanced vapor stripping as a primary treatment method, or as a stand-alone method, when the initial concentration of VOCs is low and the subsurface volume to be heated is small. In the latter case, thermophilic hydrocarbon degraders suspended in hot water are pumped into the subsurface.

Phenol. The phenol hydroxylases of thermophilic bacilli seem to be different from other phenol hydroxylases described so far (Müller et al. 1998). A thermophilic *Bacillus* sp., growing above 40°C and below 75°C, degraded up to 5 mM phenol completely at 55–70°C. Concentrations above 10 mM phenol inhibited growth. The strain also completely degraded all three isomers of 1 mM cresol, an active constituent of creosote, within 10 h, whereas toluene or xylene were not utilized as the sole carbon and energy source (Mutzel et al. 1996).

Aerobic thermophilic treatment of sewage sludge, contaminated with 4-nonylphenol (NP, a mixture of monoalkyl phenols, used in the preparation of lubricating oil additives, resins and surface active agents), was demonstrated in laboratory-scale batch reactors (Banat et al. 2000). Up to 66% reduction of 50 and 100 mg NP l^{-1} was obtained after 10 days at 60°C.

Polycyclic aromatic hydrocarbons

Little is known about degradation of polycyclic aromatic hydrocarbons (PAHs) by thermophiles. Müller et al. (1998) isolated microorganisms able to convert naphthalene, phenanthrene and anthracene under thermophilic conditions. Their studies indicate that metabolites differ significantly from those formed under mesophilic conditions. Also naphthalene degradation by a thermophilic *Bacillus thermoleovorans* at 60°C differs from the pathways known for mesophilic bacteria (Annweiler et al. 2000). In the latter case, several new metabolites (2,3-dihydroxynaphthalene, 2-carboxycinnamic acid, phthalic acid, benzoic acid) were found apart from typical metabolites well known from naphthalene degradation by mesophils.

Bioavailability of poorly water-soluble hydrocarbons, such as PAHs, is low. Solvents are frequently used to improve the solubility, but they are themselves a further source of contamination. A patent is based on the discovery that not only bioavailability of such substances increases with increasing temperature, but the oxygen transfer coefficient also rises at the same time, thereby compensating for the reduction in oxygen solubility (Markl et al. 1999). Aerobic thermophilic microorganisms, e.g. *Bacillus oleovorans* DSM 10561, can be efficiently cultivated in aqueous medium at 45°C and even above 60°C, and used for biodegradation. So far, it has been assumed that aerobic biodegradation at elevated temperature is not efficient because oxygen solubility is too low.

Chlorinated hydrocarbons

Thermophilic transformation of chlorinated hydrocarbons (chlorobenzoate, pentachlorophenol, TCE) has recently been reviewed by Müller et al. (1998). Thermophilic bacteria, such as *B. subtilis*, are used to treat soil contaminated with PCBs and dioxin; the soil is UV-irradiated and further treated in a rotary roller with a stirring paddle (Kanke et al. 1999).

Michel et al. (1995) demonstrated the mineralization of the widely used herbicide 2,4-dichlorophenoxyacetic acid (2,4-D, 17 mg kg^{-1} dw) in a laboratory-scale compost system. Mineralization of the organic matter temporally paralleled 2,4-D mineralization. After 50 days at 60°C, 47% of the 2,4-D-carbon was mineralized in the final compost, about 23% was complexed with high-molecular-weight humic acids, and about 20% were not extractable (humin fraction). Volatilization during composting was very low; and also the leachate contained only 1.3% 2,4-D-carbon.

Nitrated aromatic hydrocarbons (explosives)

The handling of explosives, such as 2,4,6-trinitrotoluene (TNT), has resulted in soil and sediment pollution at munitions factories and military facilities. Incineration is highly effective, but costly. Hater et al. (2000) have developed and patented a bioremediation strategy, suitable for treating TNT contaminations up to 3% w/w. The contaminated soil is mixed with an oxidizable carbon source and with substrates rich in microorganisms, such as manure, digester or sewage sludge. The explosives are converted in a first step (14–200 days at 21–45°C and high moisture) under anaerobic conditions into reduced derivatives (aromatic amines). Afterwards, the resulting product is mixed with compostable material and subjected to an aerobic bioremediation process (10–60 days at temperatures up to 150°C), in order to obtain a product in which the reduced derivatives are incorporated into the organic fraction of soil during natural humification. This binding of aromatic amines into the organic soil matrix is believed to be irreversible and is facilitated by anaerobic processes. There is no information about the number of thermophiles involved in the process. The stable incorporation of TNT into the humic material of composted soil has also been shown by Bruns-Nagel et al. (2000). After composting, 33% of the N-15 isotope was incorporated into the humic and fulvic acid, and 23% was present in the humin. However, the long-term stability of bound TNT residues has yet to be proven.

Williams et al. (1992) investigated the feasibility of aerated static pile composting as a suitable bioremediation technology for soils and sediments contaminated with explosives, such as TNT, HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetraazocine) and RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine). In field studies with chronically contaminated lagoon sediments, total explosives were reduced from 17,870 to 74 mg kg^{-1} over 153 days under thermophilic conditions (55°C) with NPK fertilization. TNT was reduced from 11,840 to 3 mg kg^{-1}, its half-life of 12 days was considerably lower than that obtained under mesophilic conditions (35°C). The number of heterotrophic thermophilic microorganisms increased from about 10^7 to 10^{10} cfu g^{-1} dry compost. Leachates contained low levels (µg ml^{-1}) of leachable explosives.

Soil loadings as high as 33% by mass could be incorporated during composting without affecting the thermophilic temperature during winter at daily high and low temperatures of 0°C and below.

Acidic and alkaline environments

Hydrocarbon mineralization is favored by near neutral pH values. It is common practice to add lime to bioremediate acid soils containing harmful organic compounds (Alexander 1999). However, biodegradation was reported to proceed well in aquifers at natural pH values of 4.5–5 (Norris 1994), and petroleum hydrocarbon utilizers were found in a tropical, acidic forest soil (pH varied from 4 to 6) 17 years after an extensive oil spillage (Amadi et al. 1996). Where the pH has been shifted away from neutral by manmade changes, biodegradability is likely to be impaired (Norris 1994).

Acidophilic microorganisms

Acidophiles are metabolically active in highly acidic environments, and often have a high heavy metal resistance (Norris and Johnson 1998). Heterotrophic acidophilic microorganisms are an interesting potential for the bioremediation of acidic environments that contain both heavy metals and organic compounds, such as industrial wastewater effluents or oil-polluted acidic drainage waters from metal and coal mining.

Twenty-three heavy-metal-tolerant, acidophilic heterotrophic bacteria, isolated from acidic mine effluent, metabolized a range of aliphatic hydrocarbons (such as 5 mM propane-1-ol, acetone, acetaldehyde, propanaldehyde, dodecanoic acid, hexadecanoic acid, dodecane, hexadecane, 1-chlorohexane) as the sole carbon source at pH 3. Remarkably, aliphatic organic acids, which are thought to be toxic to acidophiles (Alexander et al. 1987), were utilized as substrates for energy and growth. One isolate, closely related to the genus *Acidocella*, tolerated ethanol in excess of 250 mM (about 1.5% v/v), but could utilize high concentrations of acetic acid only if supplied in sequential small (1 mM, about 0.006% v/v) doses (Gemmell and Knowles 2000).

Stapleton et al. (1998) reported the biodegradation of aromatic hydrocarbons and PAHs in extremely acidic environments. They investigated three soil samples taken from a long-term coal pile storage basin; the pH value of areas greatly impacted by runoff from the storage basin was 2. The indigenous microorganisms oxidized about 50% of the supplied naphthalene and toluene to CO_2 and water within 24 weeks, while the extent of mineralization of phenanthrene and anthracene was only 10–20%. 16sRNA sequence analyses suggested the presence of acidophilic bacteria in the soil samples, but a microbial consortium, including eukaryotes, rather than individual acidophiles was suggested to be involved in biodegradation in this acidic environment. Initial fungal attacks on the hydrocarbons may have produced intermediates that were available for further degradation by bacteria. Whole-community nucleic acids did not hybridize to gene probes, such as *nahA*, *nahG*, *nahH*, *todC1C2*, *tomA*, that encode common degradative enzymes from neutrophilic bacteria. A mixed culture, including a fungus, a yeast and several bacteria, successfully metabolized about 27% of supplied naphthalene after 1 week at pH 3.

Genes that encode enzymes involved in biodegradation of aromatic hydrocarbons can be acquired and expressed in some heterotrophic acidophilic bacteria (Quentmeier and Friedrich 1994). A phenol-degradation encoding plasmid was successfully transferred by conjugation from neutrophilic bacteria (*E. coli* and *Pseudomonas putida*) into the extremely acidophilic heterotrophic bacterium *Acidophilium cryptum* (most members of this genus grow at pH 2–3) at high frequencies. The encoded proteins were functional, as transconjugants degraded phenol (2.5 mM). The ability to degrade phenol could not be transferred into another heterotroph acidophile (*Acidobacterium capsulatum*) or the facultatively autotrophic *Thiobacillus acidophilus*.

Alkaliphilic microorganisms

Alkaliphiles are defined as organisms that have their optimum growth rate at least 2 pH units above neutrality. Alkalitolerants are able to grow or surve at pH values above 9, but their optimum growth rate is around neutrality or less (Kroll 1990). These microorganisms have been described as producing a variety of industrially interesting extracellular enzymes, but information about their capabilities for hydrocarbon degradation is limited.

Phenol biodegradation under alkaline conditions has been demonstrated. Sarnaik and Kanekar (1995) described the potential of pseudomonads for the bioremediation of phenol from the waste effluent of the dye industry. The initial phenol concentration (244 mg l^{-1}) was completely removed within 24 h, with the simultaneous removal of color (methyl violet), and reduction in COD, TOC and ammoniacal N. Phenol biodegradation was optimal at the original alkaline pH (7.5–10.6) and 28°C. In another study, 14 isolates, able to utilize phenol (500 mg l^{-1}) at pH 10, were obtained from sediments of a highly saline and alkaline (average pH 10.5) lake in Lonar (India) (Kanekar et al. 1999). Interestingly, the indigenous microflora of the lake was never exposed to any contamination. Four of these alkaliphilic bacteria (*Arthrobacter* sp., *Bacillus cereus*, *Citrobacter freundii*, *Micrococcus agilis* and *Pseudomonas putida* biovar B) were used for bioremediation of waste effluents arising from industries that manufacture methyl violet and cumene-phenol, using phenol as a major raw material. The wastewaters had a pH of 10 and a phenol content of 370–660 mg l^{-1}, the organic load in terms of COD was 4.7–6.8 g l^{-1}. After inoculation, phenol was completely removed within 48 h at 28°C, the COD removal ranged from 11 to 56%.

The bioremediation of chlorinated phenols under alkaline conditions is of interest. Both sorption and toxicity of chlorophenols decrease as pH increases, owing to the increased conversion of the undissociated (more toxic) to the dissociated form (Kishino and Kobayashi 1995). An alkaliphilic, halophilic *Nocardioides* sp. was isolated from a contaminated site situated near the alkaline (pH 9.5–10) and highly saline (10% salinity) Alkali Lake (Oregon). Optimum growth occurred at pH 9–9.4 and 1–2.4% w/v NaCl. The strain had a unique spectrum of chlorophenol degradation. It was able to utilize 2,4-dichlorophenol and 2,4,5-trichlorophenol (these compounds are generated from agricultural biocides), and very high concentrations (up to 1.6 g l^{-1}) of the wood preservative 2,4,6-trichlorophenol as the sole energy source. The organism had a preference for phenols with chlorine substituents in positions 2 and 4 (Maltseva and Oriel 1997). Complete mineralization of 2,4-D was obtained by using three alkaliphilic, halophilic eubacteria (Halomonadaceae), isolated from a former waste storage area in the Alkali Lake (Maltseva et al. 1996). Up to 3 g 2,4-D l^{-1} was degraded in 3 days by the most active strain; optimal growth on 2,4-D was observed at pH 8.4–9.4 and 4–6% w/v NaCl. The degradation pathway is the same as that from most 2,4-D degraders from non-extreme environments.

Saline environments: halophilic microorganisms

Microorganisms requiring salt for growth are referred to as halophiles, whereas microorganisms that are able to grow in the absence as well as in the presence of salt are designated halotolerant. Extreme halophiles require generally at least 1 M NaCl (approx. 6% w/v) for growth, and grow optimally at NaCl concentrations above 3 M (Kushner 1978; Grant et al. 1998). A range of organic pollutants has been shown to be mineralized or transformed by microorganisms able to grow in the presence of salt (Oren et al. 1992; Margesin and Schinner 2001). Halophilic archaea maintain an osmotic balance with the hypersaline environment by accumulating high salt concentrations, which requires salt adaptation of the intracellular enzymes. Eubacteria are more promising degraders than archaea as they have a much greater metabolic diversity. Their intracellular salt concentration is low, and their enzymes involved in biodegradation may be conventional (i.e. not salt-requiring) enzymes similar to those of non-halophiles (Oren et al. 1992). The use of microorganisms able to degrade organic wastes in the presence of salt could prevent costly dilution to lower the salinity, or the removal of salt by reverse osmosis, ion exchange or electrodialysis before biological treatment.

There is an inverse relationship between salinity and solubility of PAHs (Whitehouse 1984). The increase in sorption of aromatic hydrocarbons (i.e. the decrease of the aqueous solubility) with an increase in salinity, as studied with pyrene in various sediment types, is a result of salting-out effects occurring in both the solution and solid (sediment organic matter) phases. The organic matter may also be salted out, becoming a better solvent for the sorbed hydrophobic aromatic molecules (Means 1995).

Petroleum hydrocarbons

Ward and Brock (1978) assumed an inverse relationship between biodegradation of petroleum hydrocarbons and salinity, because enrichment cultures from the Great Salt Lake were not able to grow on mineral oil and to mineralize hexadecane in the presence of salt concentrations above 20% w/v. The inhibitory effect of salinity at concentrations above 2.4% w/v NaCl was found to be greater for the biodegradation of aromatic and polar fractions than of the saturated fraction of petroleum hydrocarbons (Mille et al. 1991). Nonetheless, there are several reports about microorganisms able to oxidize petroleum hydrocarbons even in the presence of 30% w/v NaCl. Among such microorganisms are crude oil-degrading *Streptomyces albaxialis* (Kuznetsov et al. 1992), and an *n*-alkane (C_{10}-C_{30})-degrading member of the *Halobacterium* group (Kulichevskaya et al. 1992).

An approach to extend the capacity of efficient hydrocarbon degraders for biodegradation in saline environments has been performed by impairing the phenotype of osmotolerance to a crude oil-degrading consortium, consisting of four *Pseudomonas* strains (Kapley et al. 1999). The *E. coli pro U* (an important osmoregulatory locus) operon was subcloned and expressed into each member of the consortium. The transformed organisms could grow in the presence of 6% w/v NaCl, and the expression of the salt tolerance phenotype did not hamper hydrocarbon degradative capability. Biodegradation studies with a model petroleum mixture (500 ppm) showed that *n*-alkanes (C_{12}, C_{15}, C_{16}) had already diminished within 24 h at pH 7 and 4% w/v NaCl; and only phenanthrene, dibenzothiophene and pristane remained after 120 h. However, the ability of such constructed microorganisms to survive under in situ conditions has yet to be demonstrated.

There is little information about the effect of salinity on bioremediation of soils. An inhibitory effect of artificial salinity on mineralization of motor oil (50 g kg^{-1} soil) has been reported (Rhykerd et al. 1995). Soils were fertilized with inorganic N and P, and amended with NaCl (0.4, 1.2, 2% w/w). After 80 days at 25°C, the highest salt concentration (the conductivity of the soil water corresponded to that of an oilfield brine and was nearly four times that of seawater) had considerably inhibited oil mineralization (44% inhibition in clay loam, 20% inhibition in sandy clay loam). Thus, the removal of salt from oil-contaminated soils may reduce the time required for bioremediation. However, different results may be obtained when investigating naturally salt-containing soils, since indigenous microorganisms in such environments are expected to be salt-adapted.

The feasibility of oil bioremediation in salt marshes has been demonstrated in several studies. Salt marshes are occasionally impacted by crude oil spills. Bioremediation may be an effective method in removing oil without damage to the physically sensitive ecosystem. Seasonal variations of the mineralization potential of crude oil (520 g kg^{-1} soil) in soil samples from Lousiana salt marshes were high (Jackson and Pardue 1997). Mineralization of hexadecane (initial concentration 11.5 mg kg^{-1} soil) ranged from 0.2 to 2.4% day^{-1} (unfertilized) and from 0.81 to 3.1% day^{-1} (NP fertilized). Phenanthrene (initial concentration 1.94 mg kg^{-1} soil) mineralization rates ranged from 0.5 to 4.5% day^{-1} (unfertilized) and from 1.9 to 12.1% day^{-1} (fertilized). Nutrients reduced the lag time of hexadecane mineralization, but had little effect on the lag time of phenanthrene mineralization. In microcosm laboratory studies (700 g crude oil kg^{-1} soil), PAHs and alkanes degraded simultaneously. Low intrinsic degradation rates (0–3.9% day^{-1}) of the alkane component (C_{11}-C_{14}), but high degradation rates (8–16% day^{-1}) of the PAH fraction (naphthalene and phenanthrene) were observed (Jackson and Pardue 1999). N addition enhanced degradation of total PAHs and alkanes $>C_{20}$, while naturally present P was found to be sufficient. Contrary to this result, P significantly increased bioremediation of artificially weathered crude oil, applied at 4,620 kg oil ha^{-1} in sediments from salt marsh near Texas (Wright et al. 1997). In this study, an oleophilic fertilizer, containing N, P and a dispersant, increased oil degradation significantly, while N fertilization (urea and ammonium), with or without P, had no effect.

In another study, artificially weathered crude oil was applied to sods of marsh (soil and intact vegetation) at a dose of 2 l m^{-2} (2 mm oil thickness). The effect of three widely used bioremediation agents, a slow release NPK fertilizer, a microbial inoculation product and a soil oxidant, was investigated under controlled greenhouse conditions over 4 months (Lin et al. 1999). Inorganic nutrient addition was most effective, it significantly enhanced marsh plant growth, soil microbial populations, and oil biodegradation rate. Alkanes and aromatic hydrocarbons were reduced by 81% and 17%, respectively. Fertilization was more effective in sandy soils than in mineral soils, indicating that the soil type influenced oil bioremediation. Application of the soil oxidant or bioaugmentation had no positive effects.

Dispersants are often used to emulsify the oil slick in order to improve bioavailability and to enhance oil biodegradation. An aerobic, upflow submerged biofilter, coupled with a trickling filter, was evaluated as a means to treat emulsified diesel fuel under saline conditions (2% w/v NaCl) (Yang et al. 2000). The microbial inoculum, which tolerated up to 4% w/v NaCl, was immobilized on randomly packed plastic media particles. More than 90% TOC removal was obtained at a feed concentration of 1,000 mg TOC/l and a volumetric loading of 1.5 kg TOC m^{-3} day^{-1}. Less than 7% of the diesel fuel fed to the biofilter was volatilized, as a result of a better partitioning of the diesel fuel in the water phase accomplished by emulsification. VOCs were captured and effectively degraded in the trickling filter (68% removal in 10 s). The use of a chemical dispersant also enhanced the bioavailability of naphthalene from crude oil by the euryhaline microalga *Isochrysis galbana* (Wolfe et al. 1998). Changes in naphthalene uptake were inversely correlated with salinity, and directly correlated with temperature. The tested conditions of salinity (2.2 and 3.4%) and temperature (12 and 20°C) are encountered in Pacific waters.

Aromatic hydrocarbons (phenol)

Halophilic bacteria were employed for the treatment of hypersaline wastewaters in a periodically operated sequencing batch biofilm reactor (Woolard and Irvine 1994). A biofilm of unidentified halophiles, isolated from the Great Salt Lake, readily developed on the tubing surface and could remove more than 99% of phenol (average influent 0.1–0.13 g l^{-1}, effluent less than 0.1 mg l^{-1}) from a synthetic waste brine containing 15% w/v NaCl. A stable high-quality effluent was obtained despite fluctuations in influent phenol concentrations. The biofilm gave stable performance even when changes in salt concentration (5, 10, and 15%) were imposed; overall phenol removal rates were 113–130 mg m^{-2} h^{-1} (Woolard and Irvine 1995). Hinteregger and Streichsbier (1997) reported the suitability of a moderately halophilic *Halomonas* sp. for the biotreatment of a model saline phenolic wastewater (similar to wastewaters in oil industry) in a bubble reactor. This strain fully degraded 0.1 g phenol l^{-1} as the sole carbon and energy source within 13 h in the presence of 1–14% w/v NaCl. Phenol degradation at NaCl concentrations at or below 5% w/v was accompanied by the accumulation of an intermediate of the ortho-cleavage pathway, identified as *cis,cis*-muconic acid. The amount of this compound, which is a potentially useful raw material for new functional resins, pharmaceuticals and agrochemicals, could be raised 6-fold by increasing the phenol concentration from 0.1 to 0.6 g l^{-1}.

Two phenol-degraders, *Candida tropicalis* and *Alcaligenes faecalis*, were isolated from pristine Amazonian rain forest soil (Bastos et al. 2000). *C. tropicalis* tolerated higher concentrations of phenol and salt and a wider pH range (3–9) than *A. faecalis* (pH 7–9). The yeast degraded 1.6 g phenol l^{-1} in the presence of 15% salt within 148 h, and the bacterium utilized 1.13 g phenol l^{-1} in the presence of 5.6% salt within 200 h.

Polycyclic aromatic hydrocarbons

An unidentified halophilic archaeon degraded PAHs (acenaphtene, phenanthrene, anthracene; 500 mg l^{-1}) as well as saturated hydrocarbons (C_{14}, C_{16}, C_{18}, C_{21}, pristane) in a medium prepared with natural hypersaline water from a salt marsh (21% w/v NaCl). No growth on hydrocarbons occurred below 11% w/v NaCl (Bertrand et

al. 1990). Four bacterial strains, belonging to the genera *Micrococcus*, *Pseudomonas* and *Alcaligenes* and tolerating 7.5% w/v NaCl, could grow on 0.1% naphthalene and anthracene (Ashok et al. 1995).

Halogenated hydrocarbons

A halophilic, methane-assimilating *Methylomicrobium* sp. is able to oxidize halogenated hydrocarbons, such as TCE, in aqueous medium with 2–6% w/v salt. Such bacteria have been patented for the inexpensive and efficient bioremediation of contaminated seawater (Fuse 1998). Halophilic archaea, e.g. *Halobacterium* sp. (DSM 11147), are subjected to a patented selection process that consists of microbial adaptation to high concentrations (up to 1 mM) of various halogenated, aliphatic or aromatic hydrocarbons (Oesterhelt et al. 1998). Biodegradation can be performed in aqueous or organic media at a temperature between 20 and 60°C. A combination of cells, extracts and enzymes, eventually immobilized, is recommended for the treatment of contamination consisting of mixed halogenated compounds. Halophilic, alkaliphilic microorganisms, able to utilize chloroaromatic compounds, are described in the section about alkaliphilic microorganisms.

Deep-sea environments: barophilic (piezophilic) microorganisms

The deep sea as well as other habitats, such as deep groundwater, deep sediments or oilfields, are influenced by high pressure. Barophiles (piezophiles) are microorganisms that require high pressure for growth, or grow better at pressures higher than atmospheric pressure (Prieur and Marteinsson 1998). Little is know about the ability of deep-sea microorganisms to cope with hydrocarbons.

Pollutants with densities greater than that of marine waters may sink to the deep benthic zone, where the hydrostatic pressure is notably high. A combination of high pressure and low temperatures in the deep ocean results in low microbial activity (Alexander 1999). The rate of hydrocarbon utilization by a mixed bacterial culture that was isolated from a sediment sample at a depth of 4,940 m and consisted of *Pseudomonas*, *Aeromonas* and *Vibrio* spp., was about tenfold slower under deep-sea conditions than at ambient pressure (1 bar; 0.103 MPa): 93% of supplied hexadecane (0.3% v/v) were utilized after 4 weeks at 1 bar and 4°C, but only after 36 weeks at 500 bar (51.5 Mpa) and 4°C. The culture utilized hexadecane as sole carbon source under in situ conditions (4°C and 500 bar pressure) (Schwarz et al. 1975).

Hydrocarbon-degrading, solvent-tolerant microorganisms were isolated from deep-sea mud. A halotolerant *Flavobacterium* sp., collected from a depth of 1,945 m, was tolerant to 5% (v/v) benzene, 10% (v/v) toluene, and 10% (v/v) xylene; this strain effectively degraded *n*-alkanes in kerosene, but did not degrade PAHs. A *Bacillus* sp., tolerant to 10% (v/v) benzene, 20% (v/v) cyclohexane, and 20% (v/v) n-hexane, degraded PAHs such as naphthalene, fluorene, phenanthrene, anthracene, pyrene, chrysene and 1,2-benzopyrene (Aono and Inoue 1998).

Conclusion

A wide range of hydrocarbons that contaminate the environment, mainly due to accidental release or industrial processes, has been shown to be biodegraded (mineralized or transformed) in various extreme environments characterized by low or elevated temperatures, acidic or alkaline pH, high salinity or high pressure. This emphasizes the metabolic capacities of extremophilic microorganisms. Those adapted to more than one extreme offer a special potential for the biological decontamination of habitats where various different extreme conditions prevail simultaneously.

Most investigations on hydrocarbon biodegradation under extreme conditions have concentrated on petroleum hydrocarbon components. Comparatively little is known about the metabolism of PAHs, halogenated or nitrated compounds. In general, extreme habitats possess sufficient indigenous hydrocarbon degraders able to adapt and respond rapidly to the contamination. Hydrocarbon biodegradation can be significantly stimulated under favorable nutrient conditions.

The increasing number of patents indicates that there is a growing interest in the commercial application of extremophilic hydrocarbon degraders for the biological, environmentally friendly treatment of polluted wastewater or soil. Microorganisms useful for bioremediation must survive and be active under in situ conditions. The presence of extremophiles in polluted extreme habitats, which are adapted to the prevailing conditions and able to metabolize a wide range of hydrocarbons, indicates their usefulness for bioremediation. However, their full potential has yet to be exploited.

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