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REVIEW

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Bioremediation of hydrocarbon-contaminated polar soils

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Abstract Bioremediation is increasingly viewed as an appropriate remediation technology for hydrocarbon-contaminated polar soils. As for all soils, the successful application of bioremediation depends on appropriate biodegradative microbes and environmental conditions in situ. Laboratory studies have confirmed that hydrocarbon-degrading bacteria typically assigned to the genera *Rhodococcus*, *Sphingomonas* or *Pseudomonas* are present in contaminated polar soils. However, as indicated by the persistence of spilled hydrocarbons, environmental conditions in situ are suboptimal for biodegradation in polar soils. Therefore, it is likely that ex situ bioremediation will be the method of choice for ameliorating and controlling the factors limiting microbial activity, i.e. low and fluctuating soil temperatures, low levels of nutrients, and possible alkalinity and low moisture. Care must be taken when adding nutrients to the coarse-textured, low-moisture soils prevalent in continental Antarctica and the high Arctic because excess levels can inhibit hydrocarbon biodegradation by decreasing soil water potentials. Bioremediation experiments conducted on site in the Arctic indicate that land farming and biopiles may be useful approaches for bioremediation of polar soils.

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Introduction

Human activities in Polar Regions (continental Antarctica and regions lying north of the tree line in the Arctic) require petroleum hydrocarbons for power generation, heating and operation of vehicles, aircraft and ships. As a result of accidental spills and past disposal practices, petroleum contamination has occurred, especially around settlements including scientific bases in the Antarctic (Aislabie et al. 2004) and military bases and sites exploiting the northern oil reserves in the Arctic (Whyte et al. 1999a).

Since Polar Regions are remote, remediation of contaminated soils on or near the site of contamination is desirable. For this reason bioremediation is an attractive option. Investigations of hydrocarbon spills in Polar Regions indicate that hydrocarbon degraders, typically bacteria, are widely distributed in polar soils and their numbers are usually enhanced following hydrocarbon spillage (Atlas 1986; Aislabie et al. 2004). Furthermore, biodegradation of many of the components of petroleum hydrocarbons by indigenous cold-adapted microbial populations has been observed at low temperatures in hydrocarbon-contaminated soils (Whyte et al. 1999a, 2001; Rike et al. 2003). However, the persistence of hydrocarbons in cold soils (Atlas 1986; Aislabie et al. 2004), including light alkanes and aromatics in subsurface soils, where they are not subject to evaporation and photooxidation, indicates that in situ rates of hydrocarbon degradation are slow. Therefore, the activity of the indigenous hydrocarbon-degrading microbes is limited, likely by a combination of unfavourable conditions including low temperature and moisture, nutrient limitation, alkalinity and potentially inhibitory hydrocarbons.

In this review we describe bacteria from polar soils that degrade hydrocarbons, the factors that may limit their activity in situ and the potential for bioremediation in hydrocarbon-contaminated polar soils.

Hydrocarbon-degrading bacteria isolated from polar soils

Hydrocarbon-degrading bacteria have been readily isolated from contaminated polar soils. The bacteria are commonly psychrotolerant rather than psychrophilic, growing at low temperatures (< 10°C) but having an optimum growth temperature > 15°C. As in temperate soils, incursion of hydrocarbons results in the enrichment of heterotrophs including alkane- and aromatic-degrading bacteria that may persist at elevated levels for decades after the initial spill (Atlas 1986; Aislabie et al. 2004). This enrichment can decrease diversity in the contaminated soils compared with pristine soils (Saul et al. 2005). Although fungi such as *Phialophora* spp. and *Hormoconis resinae* (Kerry 1990; Aislabie et al. 2001) may play a role in hydrocarbon biodegradation, the cultivation of hydrocarbon-degrading microbes from cold soils typically results in the isolation of bacteria.

Alkane- and aromatic-degrading bacteria

Hydrocarbons spilled on polar soils are usually refined petroleum products such as diesel or aviation fuel, comprising aliphatic and aromatic compounds: hence the enrichment of alkane- and aromatic-degrading bacteria in contaminated polar soils. The alkane degraders frequently belong to the genera *Rhodococcus, Pseudomonas* or *Acinetobacter* (Table 1). *Rhodococcus* spp. strains 7/1, 5/1 and 5/14 isolated from Antarctic soil grew on a range of alkanes from hexane (C_6) through at least eicosane (C_{20}) and the isoprenoid compound pristane (2,6,10,14-tetramethyl-pentadecane) (Bej et al. 2000). To enhance alkane degradation, some rhodococci produce cell-associated biosurfactants that assist direct contact with solid alkanes at low temperatures (Whyte et al. 1999b). *Pseudomonas* sp. strains BI7 and BI8 isolated from Arctic soil utilized C_5 to C_{12} *n*-alkanes (Whyte et al. 1997). In addition to alkanes, many biodegradative *Pseudomonas* isolates from polar soils also degrade aromatic compounds (Table 1).

Phylogenetic analysis of 16S rRNA genes from alkane-degrading bacterial isolates indicates that *Rhodococcus* spp. from cold soils group with *R. erythropolis* or *R. fascians* (Fig. 1). Of the gram-negative alkane degraders, *Pseudomonas* isolates DhA-91 and Ps8 group with *P. fluorescens* and 5B with *P. stutzeri* (Eckford et al. 2002), although the resolution of the phylogenetic placing is less well established in this phylogenetic analysis due to limitations with the sequence quality and length available for some isolates. Of the alpha proteobacteria, DhA-95 groups with *Sphingomonas chlorophenolica* (Fig. 1).

The genes encoding the alkane hydroxylase system (typically comprising a particulate, integral-membrane alkane monooxygenase and soluble rubredoxin and rubredoxin reductase) have homologues in *Pseudomonas putida* as well as *Acinetobacter* and *Rhodococcus* spp. Hybridization of DNA extracted from Arctic and Antarctic soils with four alkane monooxygenase genotypes from *P. putida* (*Pp alkB*), *Rhodococcus* spp. (*Rh alkB1* and *Rh alkB2*) and *Acinetobacter calcoaceticus* (*Ac alkM*) revealed that *Rh alkB1* and *Rh alkB2* homologues are common in both contaminated and control soils, whereas *Pp alkB* homologues are common in contaminated soil and *Ac alkM* homologues are rare (Whyte et al. 2002; Luz et al. 2004). Furthermore, *Rh alkB1* was more prevalent in culturable psychrotolerant bacteria. Based on these hybridization results, Whyte et al. (2002) proposed that *Rhodococcus* is the predominant alkane degrader in both pristine and contaminated polar soils, whereas *Pseudomonas* may become enriched in contaminated soil and *Acinetobacter* is rare.

Aromatic-degrading bacteria isolated from polar soils typically belong to the genera *Pseudomonas* or *Sphingomonas* (Table 1). Cold-tolerant hydrocarbon-degrading *Pseudomonas* strains, including the Arctic isolates BI7 and Sag-1 and Antarctic strains Ant 9 and ST41, cluster together by 16S rRNA gene analysis with *Pseudomonas syringae* (Fig. 1), whereas others cluster with *P. fluorescens* or *P. stutzeri*. *Sphingomonas* isolates Ant 17, 44/02 and DhA-95 cluster with *S. chlorophenolica* and are related to aromatic-degrading *Sphingomonas* spp. from globally distributed sources (Aislabie et al. 2000). *Sphingomonas* Ant 20 has recently been assigned to the new species *S. aerolata*.

Pseudomonas isolates tend to degrade a narrower range of aromatic substrates than *Sphingomonas* spp. For example, *Pseudomonas* sp. strains PK4 and K319 isolated from PCB-contaminated Arctic soil grew slowly using pyrene but could not grow on naphthalene, fluorene or phenanthrene (Eriksson et al. 2002), and *Pseudomonas* sp. strains BI7 and BI8, isolated from petroleum-contaminated Arctic soil, degraded naphthalene and toluene but not fluorene (Whyte et al. 1997). In contrast, *Sphingomonas* sp. strain Ant 17 isolated from hydrocarbon-contaminated Antarctic soil degraded numerous compounds in the aromatic fraction of crude oil, jet fuel and diesel fuel (Baraniecki et al. 2002) and utilized many aromatic compounds for growth including *m*-xylene, naphthalene and its methyl derivatives, fluorene and phenanthrene.

In contrast to alkane degradation genes present in the cold-tolerant bacteria described above, the genes used for aromatic degradation by psychrotolerant and psychrophilic bacteria do not appear to differ significantly from those identified in mesophilic isolates. In fact, Whyte et al. (1996) found that catabolic genes from several aromatic-degrading psychrotolerant strains had homology to those described in mesophilic bacteria (although other isolates appeared to have novel genes). Genes for aromatic catabolism may be chromosomal or

Table 1 Hydrocarbon-degrading bacteria from polar soils and their hydrocarbon substrates for growth. Most of the bacteria listed have been identified to genus level by 16S rRNA sequence analysis

Bacterial strains	Hydrocarbon growth substrates	Reference
Alkane degraders		
Acinetobacter ADH-1	Crude oil, aromatic gas-oil, hydrogenated gas-oil, kerosene, dodecane, hexadecane, cyclohexane	MacCormack and Fraile (1997)
Arthrobacter protophormiae MTCC 688	Hexadecane	Pruthi and Cameotra (1997)
Pseudomonas DhA-91	Jet A-1 jet fuel, octane, dodecane	Yu et al. (2000)
Pseudomonas Ps 8	Jet A-1 fuel, hexadecane, pristane	Thomassin-Lacroix et al. (2001)
Rhodococcus 5/1, 5/14 and 7/1	JP8 jet fuel, C_6–C_{20} *n*-alkanes, pristane	Bej et al. (2000)
Sphingomonas DhA-95	Jet A-1 jet fuel, dodecane, pristane	Yu et al. (2000)
Rhodococcus Rho10	Jet A-1 jet fuel, dodecane	Thomassin-Lacroix et al. (2001)
Pseudomonas 5B	JP8 jet fuel, hexane	Eckford et al. (2002)
Rhodococcus 43/02	JP5 jet fuel, dodecane, hexadecane, pristane	Saul et al. (2005)
Alkane and aromatic degraders		
Pseudomonas BI7 and B18	C_5–C_{12} *n*-alkanes, toluene, naphthalene	Whyte et al. (1997)
Pseudomonas DhA-91	Jet A-1 jet fuel, octane, dodecane	Yu et al. (2000)
Pseudomonas PK4	Pyrene, dodecane, hexadecane	Eriksson et al. (2002)
Pseudomonas 30-3	JP8 jet fuel, C_8–C_{13} *n*-alkanes, toluene, *m*- and *p*-xylene, 1,2,4-trimethyl benzene	Panicker et al. (2002)
Aromatic degraders		
Pseudomonas Cam-1 and Sag-50G	Biphenyl	Master and Mohn (1998)
Pseudomonas IpA-92	Toluene	Yu et al. (2000)
Pseudomonas IpA-93	Toluene, benzene	Yu et al. (2000)
Pseudomonas Ant 5	JP8 jet fuel, NAH, 2MNAH	Aislabie et al. (2000)
Pseudomonas Ant 7	JP8 jet fuel, *p*-xylene, 1,2,4-trimethyl benzene naphthalene, 1-methyl naphthalene and 2-methyl naphthalene	Aislabie et al. (2000)
Pseudomonas 7/22	JP8 jet fuel, toluene, *m*- and *p*-xylene, 1,2,4-trimethyl benzene	Aislabie et al. (2000)
Sphingomonas Ant 17	JP8 jet fuel, *m*-xylene, 1-methyl naphthalene and 2-methyl naphthalene, dimethylnaphthalene, 2-ethylnaphthalene, fluorene, phenanthrene	Baraniecki et al. (2002)
Sphingomonas Ant 20	JP8 jet fuel, 1-methyl naphthalene, phenanthrene	Aislabie et al. (2000)
Pseudomonas K319	Pyrene	Eriksson et al. (2002)
Pseudomonas 5A	JP-8, benzene, toluene, *m*-xylene	Eckford et al. (2002)
Sphingomonas 43/03	Phenanthrene	Saul et al. (2005)
Sphingomonas 44/02	Phenanthrene	Saul et al. (2005)

plasmid-borne; for example, *Pseudomonas* sp. strain BI7, described above, carries a NAH plasmid homologue that encodes enzymes for naphthalene degradation as well as a plasmid that hybridizes to *alkB* (Whyte et al. 1997). Interestingly, the expression of NAH genes at low temperatures depends on the combination of host bacterium and plasmid (Grishchenkov et al. 2003).

Factors limiting biodegradation of hydrocarbons in polar soils

Hydrocarbon degradation is influenced by environmental conditions. In polar soils the conditions most likely to limit hydrocarbon degradation include cold and fluctuating temperatures, low nutrient levels, low moisture contents and, in some Antarctic soils, alkaline pH.

Cold and fluctuating temperatures

The climate of Polar Regions is characterized by short, cold summers, with the active layer above the permafrost typically thawing for 1–2 months every year, and extremely cold winters (Tarnocai and Campbell 2002). Mean daily air temperatures above 0°C occur only during the warmest part of the summer, and the temperatures fluctuate. In summer the surface temperatures of snow-free soils may range from below 0 to +20°C during a single day, and the temperature of soils darkened by hydrocarbon contamination may be up to 12°C warmer than adjacent control soils (Balks et al. 2002). Temperature influences the rate of microbial hydrocarbon degradation in part by affecting the physical nature of spilled oil. At low temperatures the oil viscosity is increased and the volatilization of toxic low-molecular weight compounds is reduced, thus delaying the onset of biodegradation (Margesin and Schinner 1999). Temperature also affects microbial metabolism; consequently, rates of hydrocarbon degradation in polar soils are slow. Despite these constraints, hydrocarbon degradation does occur in polar soils at low temperatures; although in situ, it is mainly restricted to the summer season when soils are thawed and water is available (Atlas 1986). However, recent reports suggest that microbial biodegradation activity does not cease at subzero temperatures (e.g., Thomassin-Lacroix et al.

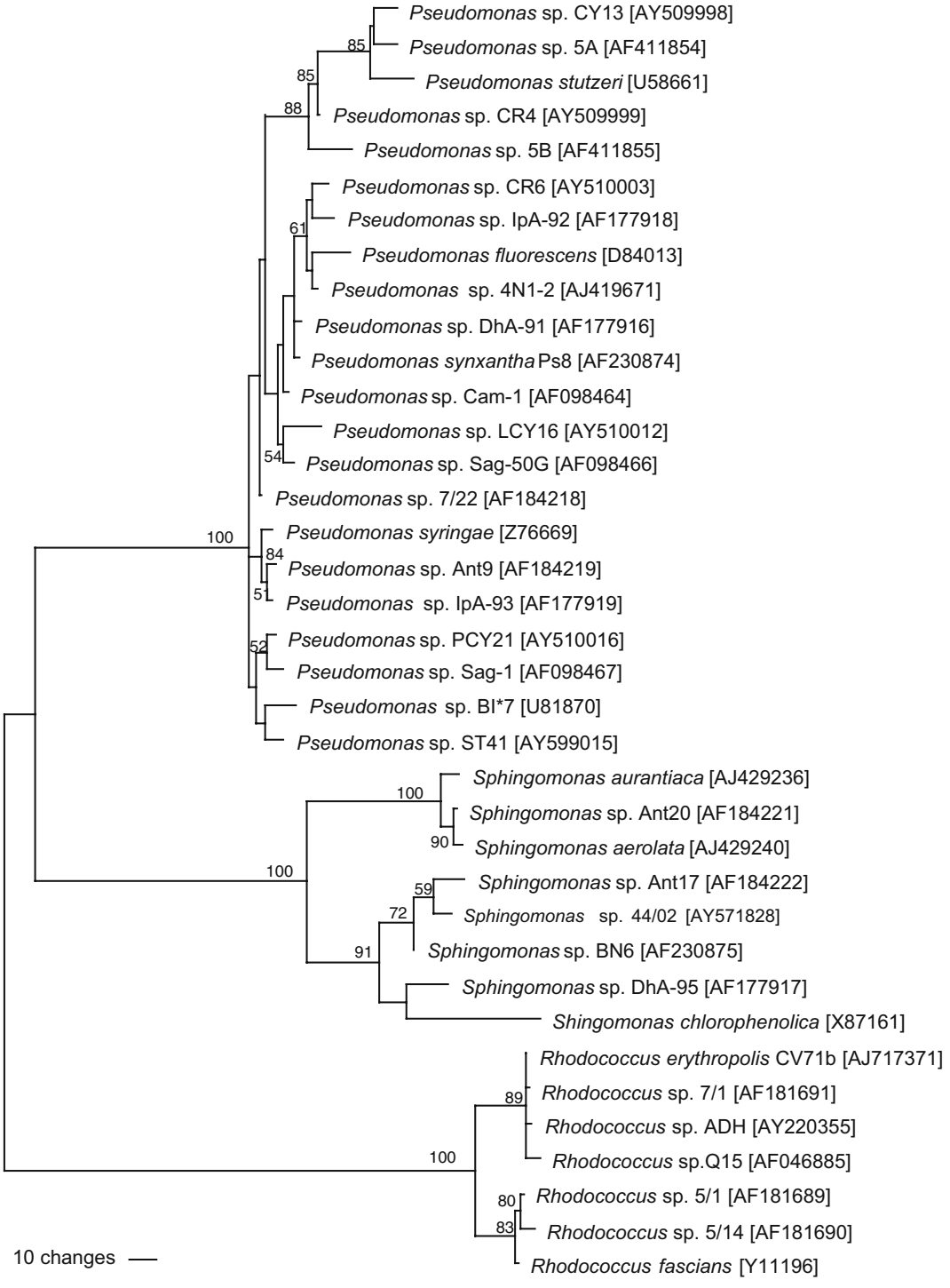

Fig. 1 Phylogenetic tree of cold-tolerant hydrocarbon-degrading *Rhodococcus*, *Pseudomonas* and *Sphingomonas*. Organisms were chosen for inclusion in the analysis on the basis of the quality and length of sequences available in GenBank. When several organisms had the same or very similar sequences (and thereby formed a tight clade), only one representative was chosen. A starting tree was constructed using neighbour-joining (NJ) with distances corrected by maximum likelihood using the HKY(85) + Γ model of evolution. This tree was then refined under the criterion of maximum likelihood by branch swapping and using the same model of evolution. All necessary parameters were estimated from the starting tree. Bootstrap values were derived by NJ with 2,000 replicates

2002; Rike et al. 2003). Rike et al. (2005) used soil oxygen depletion to infer the activity of cold-adapted microbes at a bulk soil temperature of –6°C, and hydrocarbon-degrading activity at –1 to –3°C at a contaminated permafrost site, indicating that biodegradation can occur in nominally frozen soils.

Laboratory studies have demonstrated that, although hydrocarbon mineralization occurs in soils at low temperatures, the rate and perhaps the extent of degradation are higher at elevated temperatures. ^{14}C-hexadecane was mineralized in Arctic soil at 5°C but mineralization occurred faster and to a greater extent at 23°C (Whyte et al. 1999a). In contrast, Mohn and Stewart (2000) reported that a change in incubation temperature from 7 to 22°C did not affect the extent of mineralization of ^{14}C-dodecane, but did increase the rate of mineralization and decrease the lag time. Low temperatures may affect the utilization of substrates comprising a mixture of hydrocarbons (Atlas 1986), and the combination of low temperature plus hydrocarbon substrate may affect mineralization. For example, *Sphingomonas* sp. strain Ant 17 had a lower and less well-defined optimum growth temperature when growing on jet fuel as sole carbon source than in a complex medium, and temperature had a smaller effect on the rate of ^{14}C-phenanthrene mineralization than was predicted (Baraniecki et al. 2002). These altered growth responses might result from beneficial changes to membrane fluidity caused by hydrocarbon partitioning into membranes.

In addition to low temperatures, polar soils are subject to short-term fluctuating temperatures and freeze–thaw cycles (Balks et al. 2002). In laboratory studies, hydrocarbon degradation was stimulated by alternating 24 h periods at 7 and –5°C, indicating that freeze–thaw cycles may not inhibit hydrocarbon degradation (Eriksson et al. 2001). This may result from changes to the physical matrix of the soil, making hydrocarbons more bioavailable (Eriksson et al. 2001), but additional experiments that more closely mimic in situ conditions are required to confirm this observation.

Available nutrients

Soils of Polar Regions are generally low in nutrients (Tarnocai and Campbell 2002) and the introduction of high concentrations of hydrocarbons into polar soils can further deplete available nitrogen and phosphorus when they are assimilated during biodegradation. As with temperate soils, amendment of polar soils with nitrogen and/or phosphorus can lead to enhancement in hydrocarbon mineralization (Braddock et al. 1997; Aislabie et al. 1998; Whyte et al. 1999a; 2001; Mohn and Stewart 2000). Mineralization of ^{14}C-alkanes (dodecane or hexadecane) and/or ^{14}C-naphthalene was enhanced in Antarctic soils following the addition of nitrogen as nitrate or ammonium (Aislabie et al. 1998) or by the addition to Arctic soil of commercial preparations such as 20:20:20 fertilizer, a mixture of ammonium nitrate, urea and potassium phosphate (Braddock et al. 1997; Whyte et al. 1999a; 2001). Although nitrogen is considered to be the major limiting nutrient, maximal hydrocarbon degradation occurs with supplementation of both N and P (Braddock et al. 1997; Mohn and Stewart 2000). Furthermore, P addition increased hydrocarbon degradation at 20°C but not at 10°C; presumably the higher extent of hydrocarbon degradation at 20°C led to the requirement for additional P (Walworth and Reynolds 1995).

Care must be taken, however, not to overfertilize the soils. The addition of 50–100 mg N/kg to gravelly sandy soils from Barrow, Alaska enhanced the hydrocarbon degradative activity, numbers of hydrocarbon degraders and loss of hydrocarbons, but 200 mg N/kg soil was inhibitory (Braddock et al. 1997). The total soil water potentials ranged from –200 to –1,500 kPa with increasing levels of fertilizer, and at the highest water potential microbial activity was minimal (Braddock et al. 1997). Soil microbial consumption of oxygen was halved when soil water potential reached –800 kPa due to NH_4NO_3 or NaCl amendment (Walworth et al. 1997). The optimum N level for the hydrocarbon degradation depends on soil type. Sand and loamy sand which have lower water-holding capacities were much more sensitive to overfertilization with inorganic nitrogen than silt loam (Walworth et al. 1997).

There is no consensus on the optimum C to N ratio for enhancing biodegradation of hydrocarbons in soil. Reported optimal C to N ratios range from 200:1 to 9:1 (Morgan and Watkinson 1989). Following an investigation of the relationship between soil water content and microbial response to soil N in hydrocarbon-contaminated soils, Walworth et al. (1997) recommended that the maximal N application can be calculated as the mass of N per mass of soil water rather than relative to the mass of soil or to C contaminant concentrations. Using this approach, an optimum N level of less than 2,000 mg N/kg soil water is required for sand and loamy sand soils. Subsequent investigations with Antarctic soil confirmed that the extent of ^{14}C-octadecane mineralization in soil from Casey Station incubated at 10°C peaked between 1,000 and 1,600 mg N/kg soil water (Ferguson et al. 2003). As polar soils often have low water-holding capacities, it may be difficult in practice to maintain optimal nutrient concentrations while precluding osmotic stress caused by overfertilization with inorganic fertilizers. Thus, the use of slow release fertilizers, such as cod bone meal, is being tested in cold soils (Walworth et al. 2003). Heterotrophic nitrogen fixers may also provide nitrogen for hydrocarbon biodegradation in Antarctic contaminated soils (Eckford et al. 2002).

Oxygen is not likely to be limiting in most coarse-textured dry polar soils, especially those of the Antarctic, but could be limiting in waterlogged Arctic soils (Rike et al. 2005). Under such conditions, polyaromatic hydrocarbons may degrade under nitrate-reducing conditions, as recently reported in laboratory studies with Arctic soil (Eriksson et al. 2003). The degradation of alkanes by polar microbes under anoxic conditions has not yet been investigated.

Soil moisture

Antarctic soils are typically dry, whereas Arctic soils generally have higher moisture contents, especially near the permafrost (Tarnocai and Campbell 2002). In soils, water contents of between 50 and 80% capacity are generally optimal for microbial activity (Morgan and Watkinson 1989). At lower water contents osmotic and matric forces limit the availability of water and, consequently, microbial growth. Coarse-textured soils, which are prevalent in Antarctic and the high Arctic, have low water-holding capacity. Hydrocarbon contamination can further reduce the water-holding capacity of the soils, because oil coating the surface of soil particles makes the soil more hydrophobic (Dibble and Bartha 1979).

Control of moisture levels in polar soils may be difficult. The total annual precipitation in Polar Regions is generally low, with most occurring as snow, and may not be relied upon to ensure that soil moisture is at optimum levels during in situ treatment. Covering soils for heating (Filler et al. 2001) to enhance biodegradation could also cause soils to dry out, so water may have to be added. It may not, however, always be practical or desirable to add water for in situ treatment due to the lack of fresh water in remote sites and the potential to mobilize contaminants or nutrients following water addition to uncontained hydrocarbon spills. In contrast, in some polar soils wet conditions may limit oxygen availability; hence, aeration may be required to enhance aerobic hydrocarbon degradation.

Soil pH

The pH of polar soils varies greatly depending on the chemistry of the parent material (Tarnocai and Campbell 2002). In the Antarctic, soil pH ranges from weakly acidic (pH 6) in inland soils at high elevation to highly alkaline (pH 9) in soils of coastal regions (Aislabie et al. 2004). In temperate soils, hydrocarbon biodegradation in soil has an optimum around pH 6.5–8.0 (Morgan and Watkinson 1989). Substantial hydrocarbon mineralization activity has been reported in polar soils at pH >8.8 (Aislabie et al. 1998; Whyte et al. 1999a), but adjusting soil pH from 9.4 to pH 7.4 did not enhance the activity (J. Aislabie, unpublished).

Soil pH may be modified by soil treatment, especially in mineral soils with little buffering capacity. Fertilizer may lower pH: for example, the addition of 20:20:20 fertilizer lowered the pH of coarse sand from 7.4 to 6.8 (Braddock et al. 1999). Soil pH may also decline during biodegradation due to the accumulation of acidic metabolites such as aliphatic acids produced during alkane degradation.

Bioaugmentation

An appropriate inoculum for bioaugmentation (the addition of appropriate microbes) in polar soils should contain a mixture of hydrocarbon degraders that attack the range of hydrocarbon contaminants on site. Greater success would be expected using indigenous microbes adapted to in situ polar conditions rather than allochthonous strains. Bacteria that produce emulsifiers or biosurfactants may have a selective advantage because they can counter the increased viscosity and decreased water solubility of the hydrocarbons at lower temperatures. In Antarctic soils indigenous hydrocarbon degraders are required as the Antarctic Treaty restricts the introduction of foreign microbes.

To date, the success of seeding contaminated soils with hydrocarbon-degrading microbes is ambiguous. Bioaugmentation of contaminated polar soils with microbes enriched from the same site reduced the acclimation period but did not appreciably increase ^{14}C-hexadecane mineralization or reduce the total petroleum hydrocarbon (TPH) content compared to fertilizer treatment alone (Whyte et al. 1999a). These soils probably contained enough hydrocarbon degraders prior to bioaugmentation. Likewise, Mohn and Stewart (2000) observed a decrease in lag period during the mineralization of ^{14}C-dodecane after adding both indigenous and nonindigenous bacteria, but only with a large inoculum size (10^9 colony-forming units [cfu]/g soil). The addition of a nonindigenous alkane-degrading *Acinetobacter* sp. (10^7 cfu/g soil) to Antarctic soil microcosms incubated in situ increased hydrocarbon degradation with or without nutrient addition (Ruberto et al. 2003). A parallel study using an indigenous alkane-degrading *Rhodococcus* strain reported a positive effect of bioaugmentation on biodegradation of diesel fuel (Ruberto et al. 2005). Although significant volatilization occurred during the study, inoculated microcosms showed enhanced biodegradation compared with uninoculated soil and sterilized controls. In general, however, it is questionable whether bioaugmentation of hydrocarbon-contaminated polar soils is required when there are sufficient hydrocarbon degraders in the soil; instead, amelioration of unfavourable environmental conditions seems to be more beneficial.

Application of bioremediation techniques

Because of the remoteness of many polar sites, bioremediation is often the only feasible clean-up option. There are two types of bioremediation strategies: (1) in situ, which is carried out on site without soil removal; and (2) ex situ, which involves removal and transportation of contaminated material to a different location where it is treated biologically. In situ technologies are advantageous because there is no need to remove the contaminated soil and thus costs are reduced; however, the challenge is to deliver the required amendments. The advantage of ex situ approaches, including biopiles and landfarming, is that the process can be better controlled. On the other hand, ex situ methods can be costly and

disruptive due to soil excavation. Natural attenuation may not be a satisfactory management option for most polar soils because the rate of natural degradation is so slow; hydrocarbons have been detected in surface Antarctic soils more than 50 years after spillage (Aislabie et al. 2004).

In situ treatments

Bioremediation experiments conducted in situ indicate that nutrient and/or water addition to polar soil generally enhances hydrocarbon degradation in surface polar soils (Kerry 1993; Snape et al. 2003; Delille et al. 2004a); however, impacts on subsurface soils have not been evaluated.

A study conducted on two sub-Antarctic soils revealed that the number of hydrocarbon degraders increased by two orders of magnitude during the first month following application of the slow release fertilizer Inipol EAP-22 (Elf Atochem). However, the stimulation was less marked over time, with fertilizer having a greater effect in a desert soil than a vegetated soil (Delille et al. 2004a). Hydrocarbon analysis indicated that straight chain alkanes were degraded faster in treated plots, whereas fertilizer had little effect on aromatic degradation (Coulon et al. 2004). Some plots were covered with black sheet plastic resulting in a 2°C increase in soil temperature and an increase in alkane degradation, but not aromatic degradation, in the desert soil (Delille et al. 2004b). Furthermore, the Microtox® assay revealed that the toxicity of residual hydrocarbons had not been reduced (Coulon et al. 2004). The addition of nutrients (either Inipol EAP-22 or fish compost) to ornithogenic soils contaminated with diesel or crude oil did not increase the numbers of hydrocarbon-degrading bacteria in ornithogenic soil, unlike the desert soil (Delille et al. 2003). The addition of controlled-release nutrients and water did enhance hydrocarbon degradation in soils at Casey Station, Antarctica; after three summer seasons hydrocarbons were reduced from 23,000 to 2,500 mg/kg, although much of the loss was attributed to volatilization (Snape et al. 2003).

Biopiles

A biopile or biocell is an aboveground mound of soil similar to a compost pile, often constructed on an impermeable liner and fitted with aeration pipes; it can be amended with nutrients and/or moisture to stimulate biodegradation, and may be mixed mechanically. Ex situ biopiles have been used successfully for the bioremediation of diesel-contaminated Arctic soils using combinations of biostimulation (heating, nutrients and aeration) and bioaugmentation. Active (Filler et al. 2001) and passive heating (by covering biopiles with clear plastic) increased in situ soil temperatures from 1 to 5°C (Mohn et al. 2001) and increased hydrocarbon biodegradation rates. Importantly, biodegradation has also occurred in soil when daily air temperatures were below 0°C, hence, effectively extending the season of treatment (Thomassin-Lacroix et al. 2002). Hydrocarbon analysis of Arctic soil biopiles constructed with layers of fertilizer revealed enhanced alkane degradation ($>C_{12}$) in surface and subsurface soils, whereas volatilization was a major mechanism for the loss of aromatic compounds from the surface. The loss of linear alkanes was more strongly affected by fertilizer addition than branched alkanes (Braddock et al. 1999).

Bioaugmentation of biopiles with enrichments of cold-adapted microbes has yielded variable results in the Arctic (Mohn et al. 2001; Thomassin-Lacroix et al. 2002). Mohn et al. (2001) reported enhanced degradation in biopiles during the summer after inoculation with a cold-adapted, mixed microbial culture derived from soil, whereas Thomassin-Lacroix et al. (2002) saw no increase in biodegradation after inoculation with an enrichment culture originating from the site. Nutrient amendment, however, consistently improved bioremediation (Mohn et al. 2001; Thomassin-Lacroix et al. 2002).

Landfarming

Landfarming or land application is a larger-scale land treatment method where thin layers of excavated contaminated soil are spread on ground that is amended with nutrients and/or moisture and tilled to achieve aeration. Landfarming is a simple low cost ex situ bioremediation method that has been utilized in Polar Regions (McCarthy et al. 2004). Recently, 3,600 m^3 of sandy soil was successfully treated at field scale by landfarming on site at Barrow, Alaska (McCarthy et al. 2004). Soil was amended with a mixture of fertilizers (monoammonium phosphate and urea) applied manually. The site was managed with intensive tilling and selective fertilization to exploit the short treatment season, and the soil moisture content (3–6%, near field capacity) was maintained by light precipitation. Soil concentrations of diesel range organics reached target concentrations ahead of schedule, although the proportion of losses due to biodegradation versus evaporation was not determined.

Conclusions

Despite the environmental extremes of polar soils, spillage of hydrocarbons can stimulate in situ selective enrichment of hydrocarbon-degrading bacteria in surface soils. The bacteria reported so far are usually psychrotolerant, probably reflecting summer in situ temperatures that may range from below 0°C to about 20°C. The optimal temperature for hydrocarbon degradation and the influence of freeze–thaw cycles have yet to be resolved. Clearly, however, nutrient addition is

required for effective bioremediation. Given the low temperatures in subsurface soils and the short season during which soils are thawed (1–2 months) there is a need to control temperature, nutrient levels and moisture to optimize degradation rates. Hence, ex situ bioremediation is likely to be the strategy of choice for remediation of hydrocarbon-contaminated polar soils.

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