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Review Article

Bioremediation of Petroleum Hydrocarbon in Antarctica by Microbial Species: An Overview

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ABSTRACT

The increase of anthropogenic activities and growth of technology in Antarctica is fuelled by the high demand for petroleum hydrocarbons needed for daily activities. Oil and fuel spills that occur during explorations have caused hydrocarbon pollution in this region, prompting concern for the environment by polar communities and the larger world community. Crude oil and petroleum hydrocarbon products contain a wide variety of lethal components with high toxicity and low biodegradability. Hydrocarbon persistence in the Antarctic environment only worsens the issues stemming from environmental pollution as they can be long-term. Numerous efforts to lower the contamination level caused by these pollutants have been conducted mainly in bioremediation, an economical and degrading-wise method. Bioremediation mainly functions on conversion of complex toxic compounds to simpler organic compounds due to the consumption of hydrocarbons by microorganisms as their energy source. This review presents a summary of the collective understanding on bioremediation of petroleum hydrocarbons by microorganisms indigenous to the Antarctic region from past decades to current knowledge.

Keywords: Antarctic region, bioremediation, fuel spills, indigenous microorganisms, petroleum hydrocarbons

INTRODUCTION

Accidental spills of oils and fuels in Antarctica is particularly sensitive as the environment is near pristine, causing pollution and its effects to be more apparent. Cold temperatures also

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cause the natural processes that help remove contamination in other parts of the world to happen far more slowly, making it possible for contaminants to build up (AMAP 1998; Det Norske Veritas, 2003). For instance, hydrocarbon compounds can take decades to completely mineralise in this region, while only months are needed for compounds to be removed in temperate regions. Common spills are typically of kerosene and the Special Antarctic Blend diesel. Fuel spills have been acknowledged as being the most common source of pollution; thus have a great potential to cause the greatest environmental harm in and around the continent. Such spills have been reported to occur mainly near the Antarctic research stations and military bases, mainly due to the poor management or regulation of such pollution (Aislabie, Balks, Foght, & Waterhouse, 2004). Fortunately, due to the Antarctic Treaty prohibition on oil exploration and exploitation (Rothwell & Davis, 1997), petroleum hydrocarbon contamination manages to remain at controlled levels to a certain extent compared with other cold regions.

Attempts to clean up Antarctic polluted sites have been done using both physical and chemical methods. However, the estimated budget for the application of physical machinery would be large due to the harsh environment of the Antarctic, while chemical application should be critically considered as the introduction of chemicals may add to the risks of environmental pollution. Bioremediation, which basically manipulates the utilisation of hydrocarbon by microbial species as their energy source, is highly recommended (Das & Chandran, 2011; Jesus, Peixoto, & Rosado, 2015). Successful attempts of Antarctic bioremediation have been widely reported and reviewed several times to date (Azubuike, Chikere, & Okpokwasili, 2016; McWatters et al., 2016). However, the effectiveness of degradation of hydrocarbons by native microorganisms has been reported in laboratory studies but not on a pilot scale.

In this paper, we review the composition of petroleum hydrocarbons and their toxicity; the physical management of oil spill site; hydrocarbon degradation by microbial species; factors that affect the rate of biodegradation; and new bioremediation prospects in Antarctica. The purpose of this review is to fill the gap in the literature in order to direct relevant scientific research on areas to be explored in the future.

PETROLEUM HYDROCARBONS – COMPOSITION, FATE AND TOXICITY

Petroleum is a complex blend consisting of both aliphatic and aromatic hydrocarbons. Small amounts of non-hydrocarbons such as resin and asphalt can also be found in petroleum mixture. Crude oils can be divided into paraffinic, asphaltic and mixed crude oils (WHO, 1982). Paraffins (aliphatic hydrocarbons), wax and high-quality oils belong to the paraffinic crude oils group. Kerosene and naphtha are the lightest among paraffinic fractions. High viscous lubricating oils and cycloaliphatics can be grouped into asphaltic crude oils. These petroleum solvents are the product of crude oil distillation and are generally characterised by boiling point ranges. Lubricants, greases and waxes are high boiling-point portions of crude oils, whereas the heaviest solid fractions of crude are the residuals or bitumen (ATSDR, 1999).

Petroleum hydrocarbons are extensively used in the Antarctic as a source for heating, transportation and generating electricity (Aislabie, Saul, & Foght, 2006). Because of the broad usage of hydrocarbons, contamination of these pollutants is likely to occur (Mohn & Stewart, 2000; Ruberto, Vazquez, Lo Baldo, & Mac Cormack, 2005). As crude oils are made up of many such components, they have the potential to become environmental contaminants, depending on the nature of release and fate of hydrocarbons in the environment. The structure and components of hydrocarbons do change and transform physically, chemically and biologically directly after crude oil is released into the environment, an action that significantly affects their potential impacts. The succession of transformations may be due to evaporation, dissolution, emulsification, dispersion, adsorption, sedimentation, biodegradation and photo-oxidation, with carbon dioxide and water as the ultimate products (Montagnolli, Lopes, & Bidoia, 2015).

The degradation of hydrocarbons in the Antarctic environment can be divided into two different processes, which are the soil systems and the aqueous systems. According to Aislabie et al. (2004), hydrocarbon spills on Antarctic soils generally undergo naturally occurring processes that decrease the mass of the pollutants. Although mechanisms such as dispersion, volatilisation and microbial degradation typically occur at most spill sites, degrees of the hydrocarbon mass reduction depend on the type and concentration of the spilled fuels and also the soil characteristics itself. Hydrocarbons with high vapour pressure such as jet fuel and MoGas tend to volatilise quickly after spills in Antarctic soil but due to their low viscosity, they are mobile and are able to migrate down through the unfrozen soil active layer (Webster, Webster, Nelson, & Waterhouse, 2003). Meanwhile, there is a limit to the downward movement due to the ice-saturated layer, causing an emergence of oil pools of the less volatile and more viscous engine and lubricating oils (Chuvilin, Naletova, Miklyaeva, Kozlova, & Instanes, 2001a). The freeze-thaw cycle may also influence the hydrocarbon movement in Antarctic soils. It is based on the implication of the melting of the active frozen layer and snow, which make the mobilisation of hydrocarbon compounds in downward movements easier (Chuvilin et al., 2001a). Contamination of the offshore marine environment due to surface runoff has also been reported (Kennicutt, McDonald, Denoux, & McDonald, 1992).

The fate of crude oil in marine ecosystems does not deviate much from oil degradation in soil ecosystems as both ultimately undergo total biodegradation by microbial species (Das & Chandran, 2011). In marine ecosystems, oil is likely to spread on the surface water when spilled and almost directly subjected to many modifications called the weathering process. The weathering process of oil is mainly due to the evaporation of low molecular weight hydrocarbons, dissolution of water soluble components, emulsification of oil droplets, photo-oxidation and lastly, biodegradation (Harayama, Kishira, Kasai, & Shutsubo, 1999; National Research Council, 2003). The succession of the reduction of oil pollutants in marine systems can be stated in the following order: evaporation of low boiling point *n*-alkanes and aromatic compounds > photochemical modification under sunlight (during summer season) > dissolution and dispersion of oil droplets by wave action > emulsification of the oil 'chocolate mousse' > formation of tar balls from petroleum heavy residues > sinking and degradation of tar balls by microbial species at the sediments of the sea (Harayama et al., 1999).

Wang and Bartha (1990) stated that the contamination can cause risks for humans and other living organisms if spilled fuel reaches groundwater reservoirs and water bodies. Compounds derived from petroleum tend to be toxic to a small population of animals and plants in the Antarctic region. Moreover, due to their persistence in the cold environment, they have a long-term carcinogenic potential that could affect this populace (Montagnolli et al., 2015). Long-term toxicity effects associated with petroleum substances represent a major concern. Different types of hydrocarbon differ in their toxicity upon the local habitat. Generally, the longer the carbon chain and the higher the number of benzene rings possessed by hydrocarbons, the higher the toxicity (Montagnolli et al., 2015). The order of toxicity also tends to correspond to hydrocarbon susceptibility to microbial degradation. The order of hydrocarbon toxicity/susceptibility to microbial degradation can be arranged in decreasing order as follows: polycyclic aromatics hydrocarbons (PAHs) > benzene > heavy oil > light oil > aromatic hydrocarbons > alkanes (Atlas & Bragg, 2009; Paixão et al., 2007).

Polycyclic aromatic hydrocarbons (PAHs), the higher molecular weight aromatic hydrocarbons, are the most toxic components in a hydrocarbon mixture. The acute effects of PAHs on human health will vary due to the degree of exposure, PAHs quantity during exposure, the level of toxicity of PAHs and the route of exposure (ACGIH, 2005). PAHs have also been reported to cause skin irritation and inflammation, nausea and vomiting in low concentrations (Kim et al., 2013). Long-term effects are more severe, such as the production of cancer cells in the vital organs, gene mutation and cardiopulmonary mortality (Abdel-Shafy & Mansour, 2016; Armstrong, Hutchinson, Unwin, & Fletcher, 2004; Kuo, Hsu, & Lee, 2003). Although the probability of health effects due to PAHs is small and remains understudied in the Antarctic environment, deterioration of health can be damaging as the chronic effect of hydrocarbons is more prone in cold temperature. The toxic effects of petroleum hydrocarbons (including in temperate region) on humans and other living organisms are listed in Table 1 and 2.

Table 1
Toxic effects of different types of hydrocarbon on animals and plants

Hydrocarbon	Toxic effect	Reference(s)
Automated diesel	Changes in CD-1 mice biological activity	Singh et al. (2004)
Gasoline vapours	Impaired the levels of monoamine neurotransmitters in CD-1 mice's brain	Kinawy (2009)
Crude oil	Induces reproductive cytotoxicity confined to the differentiating spermatogonia compartment in rats	Obidike, Maduabuchi, & Olumuyiwa (2007)
PAHs	Impaired glucose metabolism and changes in tricarboxylic acid (TCA) cycle intermediates in earthworm *Lumbricus rubellus*	Jones, Spurgeon, Svendsen, & Griffin (2008)
Petroleum effluent	Low reproduction of earthworm *Eudrilus eugebiae*	Oboh, Adeyinka, Awonuga, & Akinola (2007)
TPH	Reduced seed germination and root growth of *Festuca arundinacea* (tall fescue) and *Euclaena mexicana* (Corn grass)	Tang, Wang, Wang, Sun, & Zhou (2011)
Special Antarctic Blend (SAB) diesel	Reduction of photosynthetic efficiency of green and Antarctic moss	Nydahl, King, Wasley, Jolley, & Robinson (2015)
Diesel fuel	Inhibition of shoot and root growth of *Colobanthus muscoides* (sub-Antarctic moss)	Macoustra, King, Wasley, Robinson, & Jolley (2015)

Table 2
Toxic effects of different types of hydrocarbon on human health

Hydrocarbon	Toxic effect	Reference(s)
Crude petroleum	Chronic cough and phlegm	Rodriguez-Trigo et al. (2010)
Crude oil	Lower respiratory tract symptoms	Zock et al. (2007)
Crude oil	Increased rates of bronchial hyperresponsiveness	Zock et al. (2009)
Crude oil	Persistent respiratory symptoms	Zock et al. (2012)
Volatile organic compounds (VOCs)	Elevated rates of genotoxic damage	Aguilera et al. (2010)
PAHs	Elevated toxicity in reproductive organs	ATSDR (1995)
	Skin and lung cancer	ATSDR (1995)
	Chronic ingestion due to contaminated food	Aguilera et al. (2010)
Diesel exhaust	Irritation to eyes, nose, throat and lungs and increase in the frequency or intensity of asthma attacks	OEHHA (2001)
	Potential of carcinogens in humans	IARC (2012)

The physical characteristics of oil itself can lead to health and environmental disturbance. Deposits of oils tend to accumulate in sewage pipe walls and can cause operational and physical damage, leading to bad odours and the promotion of chemical and microbiological corrosion. These oil clumps also stick onto pipes and ultimately cause filter blockage, which can create pathogenicity issues (Xu & Zhu, 2004). Thick black oils also tend to stick to the feathers of penguins and other birds in the event of an oil spill. This ruins the waterproof coating on the birds' feathers, which could lead to their death due to freezing. Oil that they may swallow is poisonous to them (Kalman & Johnson, 2007).

MANAGEMENT OF OIL SPILL SITES

The Antarctic Treaty Consultative Meeting held in 1998 agreed on the decision to adopt guidelines in the management of oil spills that included measures such as fuel oil handling in scientific stations, spill prevention, control of fuel oils, oil spill contingency planning and reporting of oil spills (ATCM, 1998). Although fuel spills still happen, one might expect their occurrence and frequency to reduce with improvements in both infrastructure and practical applications (Aislabie et al., 2004).

Since then, various attempts to remove pollutants from the Antarctic environment via physical, chemical and biological methods have been established. Treatment of hydrocarbon-polluted Antarctic soils are frequently reviewed (Camenzuli & Freidman, 2015; Jesus et al., 2015) as petroleum hydrocarbon contamination in Antarctica is typically focussed around both operating and abandoned research stations (Curtosi, Pelletier, Vodopivez, & Mac Cormack, 2007; Snape, Morris, & Cole, 2001).

The initial response to a fuel spill is to remove mechanically as much contaminated soil as possible, which includes the underlying snow or ice. These contaminated pieces are then shipped back to the home country to be disposed of. Although this method is still the prevalent way to remove hydrocarbon pollutants, the fate of the spilled fuel and the impact of removal on the ecosystems cannot be estimated directly (Aislabie et al., 2004). Moreover, this kind of methodology may add up to the original damage towards the environment such as altered streamflow, soil shrinkage, land slumping (due to melting of permafrost) and add to contaminants in other places (Campbell, Claridge, & Balks, 1994; Perelo, 2010). Due to this, more refined approaches are required for both preservation of local environmental conditions and for ethical reasons.

As demands for the eco-friendly technique are rising, alternative remediation methods such as bioremediation are highly proposed and currently established for the Antarctic. Aislabie et al. (2004) stated that bioremediation is gradually regarded as a suitable remediation technology for polluted sites in Antarctica. Successful bioremediation attempts in the clean-up of the Exxon-Valdez oil spill in 1989 (Atlas & Bragg, 2009) also sparked remarkable interest in using bioremediation in cold environments. The accomplishment of oil spill bioremediation varies according to the ability to ascertain and maintain settings that boost oil biodegradation rates in the contaminated environment (Das & Chandran, 2011). In general, fuel spill bioremediation can be separated into two forms: biostimulation and bioaugmentation.

In biostimulation, indigenous soil microbial populations are stimulated through the addition of relevant nutrients and the characteristics of the environmental settings such as pH, oxygen content and also temperature. Successful biostimulation has been reported in Antarctica (Delille, Pelletier, Delille, & Coulon, 2003; Dias et al., 2012). Meanwhile, bioaugmentation encompasses the addition of a pre-adapted microbial strain/consortium or introduction of genetically-engineered microorganisms to particular contaminants present on the site (Tyagi, Da Fonseca, & De Carvalho, 2011). This method has been proposed in Antarctic soils with positive outcomes (Ruberto, Vazquez, & Mac Cormack, 2003; Ruberto et al., 2009).

MICROBIAL DEGRADATION OF PETROLEUM HYDROCARBONS

Bioremediation has been characterised as a pleasant method for petroleum hydrocarbon removal using the application of microbes as it is easy to maintain, applicable over wide areas, economical and leads to complete mineralisation of the pollutants. However, as the Antarctic Treaty prohibits the importation of foreign organisms to the Antarctic environment, only local microbes are required for the bioremediation purposes (Aislabie, Foght, & Saul, 2000).

According to the definition given by Morita (1975), cold-adapted microbes can be differentiated as cold-loving (psychrophilic) microbes and cold-tolerant (psychrotolerant) microbes on the basis of their cardinal temperature. Psychrophilic microbes can be described as a microbial group that achieved the ideal growth temperature of 15°C and below, with growth inhibition above 20°C. In contrast, psychrotolerant microbes can grow over a broad range of temperatures, exhibiting the fastest growth rates above 20°C (Lo Giudice, Bruni, De Domenico, & Michaud, 2010).

Bacteria are the most prevalent species in petroleum hydrocarbon degradation as they play a major role in degrading hydrocarbon pollutants. *Rhodococcus, Pseudomonas* and *Sphingomonas* are among the bacterial group that are commonly isolated from the Antarctic environment polluted by hydrocarbons (Ruberto et al., 2005; Aislabie et al., 2006). They are the most diverse group of microorganisms that is strongly reliable in hydrocarbon degradation in Antarctica and has been reported to degrade both alkanes (Bej, Saul, & Aislabie, 2000; Shukor et al., 2009) and/or aromatic hydrocarbons (Aislabie et al., 2000) aerobically as shown in Table 3. *Rhodococcus* spp. are one of the promising group in hydrocarbon degradation due to their outstanding qualities. Owing to the multiple alkane hydroxylase systems in them, the extent of alkane compound mineralisation is broad (Aggarwal, Dawar, Phanindranath, Mutnuri, & Dayal, 2016; Nie et al., 2014; Whyte et al., 2002). Production of biosurfactants by *Rhodococcus* spp. isolated from the Antarctic have also been reported by Gesheva, Stackebrandt and Vasileva-Tonkova (2010) and Malavenda et al. (2015). The combination of these particular properties together with their tolerance for cold environments definitely establishes them as an effective tool for Antarctic soil remediation. Another bacterial group that has shown a capability for utilising hydrocarbons as carbon and energy sources are *Pseudomonas* spp. and *Sphingomonas* spp. Comparable to *Rhodococcus* spp., this group of bacteria also has several distinctive properties such as biosurfactant production for the former (Pacwa-Plociniczak, Plaza, Poliwoda, & Piotrowska-Seget, 2014; Santa Anna et al., 2002) and the ability to degrade both simple aromatic hydrocarbons and PAHs that have low susceptibility towards microbial degradation (Aislabie et al., 2000; Ma, Wang, & Shao, 2006). Based on the collected reports on the hydrocarbon-degrading ability of microbial isolates, hydrocarbon degraders show a high prevalence of psychrotolerant rather than psychrophilic microbes. Lo Giudice et al. (2010) asserted that this finding may suggest the temperature values and fluctuations that characterise the cold habitat of the microbes' origin. Hence, further efforts should be aimed at isolating pure psychrophilic hydrocarbon degraders, especially those from permanent cold environments.

The mechanistic approach of bacteria in degrading petroleum hydrocarbon is commonly predicted under aerobic degradation, as it is the most rapid and offers complete degradation (Das & Chandran, 2011). Enzymes play a major role in biodegradation of recalcitrant compounds. The process of bioremediation basically depends on microbial enzymes that attack the contaminants, thus converting them into harmless compounds such as carbon dioxide and water molecules. The degradation of petroleum hydrocarbons can be mediated by specific enzyme systems. Even though hydrocarbon degradation in the Antarctic by the anaerobic process has been reported (Powell, Ferguson, Snape, & Siciliano, 2006), aerobic degradation is the prevalent mineralisation pathway chosen mostly by bacteria. Monooxygenases, which are the alkane-activating enzymes, incorporate O_2 as a reactant for the activation of alkane molecules. Another group of enzymes, hydroxylases, initiates oxidation of alkane chains (Rojo, 2009). One of the most commonly mentioned hydroxylase systems is the Cytochrome P450 enzyme system, which is able to hydroxylate large numbers of compounds (Sekine et al., 2006). These enzymes act as the key component in initiating hydrocarbon degradation.

The subsequent pathway of alkane degradation after the involvement of hydroxylases is dependent on the mechanism of the bacteria itself. The most generic one is the yielding of *n*-alcanols by alkane hydroxylases, which are then continued with the oxidation of membrane-bound alcohol dehydrogenase to form *n*-alkanals (Harayama et al., 1999). After transformed subsequently to fatty acids, the products are likely to enter the fatty acid metabolism and eventually produce the ultimate products, carbon dioxide and water.

Meanwhile, Harayama et al. (1999) stated that cycloalkane degradation involves the co-oxidation mechanism as formation of cyclic alcohol and ketones has been observed. A monooxygenase then introduces an oxygen atom into the cyclic ketone causing the cyclic ring to be cleaved (Morgan & Watkinson, 1994). The catabolic pathways for aromatic compounds can be achieved through several pathways. Although different microbial species utilise different pathways for mineralisation of these compounds, hydroxylases and monooxygenases still play the major role in the whole picture (Johnson & Olsen, 1997). In general, the biodegradation of PAHs is initiated by dihydroxylation of one of the polynuclear aromatic rings, followed by cleavage of the dihydroxylated ring. Both hydroxylation is catalysed by a multi-component dioxygenase comprising a reductase, a ferredoxin and an iron sulfur protein, while ring cleavage is normally catalysed by an iron-containing meta-cleavage enzyme. The carbon outline produced by the cleavage reaction is then disassembled, before cleavage of the second aromatic ring (Harayama, Kok, & Neidle, 1992; Saito, Iwabuchi, & Harayama, 1999).

The uptake mechanism of alkanes varies depending on the bacterial species, the molecular weight of the alkanes and the physico-chemical characteristics of the polluted surroundings (Wentzel, Ellingsen, Kotlar, Zotchev, & Throne-Holst, 2007). Low-molecular weight alkanes can be uptaken directly, but for medium- and long-chain *n*-alkanes, microbes may gain contact to the compounds by adhering to hydrocarbon precipitates or by surfactant production (Rojo, 2009). Biosurfactants are surface-active biological compounds produced by a wide variety of microorganisms. One of the advantages of biosurfactants in bioremediation is that they have the ability to improve solubilisation and removal of contaminants (Muthusamy, Gopalakrishnan, Ravi, & Sivachidambaram, 2008). In general, biosurfactants act as emulsifying agents by decreasing the surface tension and forming micelles, which encapsulate the contaminants before they are taken up and degraded by cells.

The existence of filamentous fungi has been reported in Antarctic cold environments, while discovery of hydrocarbon-degrading yeast from the Antarctic has never been reported to the present day. Several hydrocarbon-degrading fungi isolated from Antarctica are listed in Table 4. However, Margesin and Schinner (1997) have reported on a yeast strain, identified as *Yarrowia lipolytica*, that effectively degraded hexadecane and dodecane isolated from the Alpine cold-habitat. Although fungi show capability for utilising hydrocarbons, information on their mechanistic approach and full potential is limited compared to that on bacteria.

Table 3
List of several Antarctic hydrocarbon-degrading bacteria

Strain	Substrate degraded	Isolation place	Reference(s)
Rhodococcus DM1-21	C_{12}-C_{30}, crude oil, gas oil	Marambio station	Ruberto et al., 2005
Rhodococcus B11/B15	Diesel oil	Terra Nova Bay, Ross Sea	De Domenico et al., 2004
Rhodococcus JG-3	N/A (grown on TSA/TSB)	Dry Valleys	Goordial et al., 2015
Rhodococcus sp.	Crude oil, diesel oil	South Shetland Islands	Malavenda et al., 2015
Pseudomonas J3	Diesel oil	Jubany station	Shukor et al., 2009
Pseudomonas Ant 9	xylene, benzene, naphthalene	Scott Base	Aislabie et al., 1998
Pseudomonas LCY16	Naphthalene, phenanthrene	Great Wall station	Ma et al., 2006
Sphingomonas Ant 17	Naphthalene, phenanthrene	Scott Base	Baraniecki et al., 2002

*N/A - not available

Table 4
List of several hydrocarbon-degrading fungi from Antarctica

Strain	Substrate degraded	Isolation place	Reference(s)
Arthroderma sp. 1	Monoaromatic hydrocarbon	Macquarie Island	Ferrari et al., 2011
Pseudeurotium bakeri	Diesel oil	Macquarie Island	Ferrari et al., 2011
Mortierella sp. HC8D	Dodecane	Rothera research station	Hughes et al., 2007
Phialophora sp.	N/A (#)	Scott Base, Marble Point	Aislabie et al., 2001
Chrysosporium sp.	N/A (#)	Scott Base, Marble Point	Aislabie et al., 2001
Alternaria sp.	N/A (#)	Scott Base, Marble Point	Aislabie et al., 2001

*N/A - not available, # - isolated from hydrocarbons-contaminated sites

FACTORS AFFECTING HYDROCARBON DEGRADATION IN ANTARCTICA

The Antarctic environment is known for its harsh environment – extremely cold weather, strong winds and brutal storms (Mishra, Yadav, Sharma, Ganju, & Singh, 2014). These conditions become limiting factors in hydrocarbon degradation in this region. Among the factors that affect the hydrocarbon biodegradation rate are temperature and nutrient availability.

Temperature is one of the most important aspects in determining the success of biodegradation. Ma et al. (2006) agreed that low temperatures in Antarctica are the main limiting factor of hydrocarbon biodegradation, as physical nature and composition of spilled oil are more prone to modifications in such temperature. According to Margesin and Schinner (1999), due to cold temperatures, oil tends to increase in viscosity, thus causing hydrocarbon volatilisation to be reduced and slowing down the mineralisation process. As the rate of reaction is thought to follow the Arrhenius relationship, biodegradation will be assumed to decrease as the temperature decreases, which is a disadvantage of the Antarctic cold environment. However, contaminants can be reduced in the Antarctic cold environment as bioremediation treatments are usually done and proposed in the summertime, when temperatures are higher, where soils are slightly thawed and water is more accessible (Atlas, 1986). Hydrocarbon degradation at low temperatures has been investigated in the laboratory in the range of 4-30°C (Aislabie et al., 2006; Muangchinda et al., 2015), but there have been no reports on degradation in subzero temperature.

Necessary nutrients are also one of the important factors in dealing with biodegradation of petroleum hydrocarbon. Fuel spills on Antarctic soil (or water bodies) can raise the levels of soil organic carbon, which may either serve as substrates for microbial growth or be growth-retardant due to toxic effects (Bossert & Bartha, 1984). However, the increased proportions of carbon in the environment reduce the availability of other nutrients like nitrogen and phosphorus to the microorganisms, thus disturbing nutrient balance in the Antarctic environment. Due to low nutrient levels in polluted Antarctic sites, several attempts have been made to increase nutrient concentration such as addition of oleophilic fertilisers to sub-Antarctic soils (Coulon, Pelletier, St. Louis, Gourhant, & Delille, 2004); optimisation of the biostimulation strategy (Alvarez, Lo Balbo, Mac Cormack, & Ruberto, 2015); and supplementation of nutrients/water (Stallwood, Shears, Williams, & Hughes, 2005).

Furthermore, availability of oil waste and oxygen in the environment to microorganisms does influence the rate of biodegradation. For the former, Barathi and Vasudevan (2001) stated that hydrocarbon compounds tend to bind to soil particles, causing the degradation and removal of hydrocarbons to be harder. These problems, however, can be countered in certain ways – usage of biosurfactant-producing microorganisms, biopiling and soil heating. Atlas (1991), meanwhile, stated that the availability of oxygen in soils, sediments, and aquifers are often limiting and reliant on the type of soil and whether the soil is clogged, thus disturbing the aerobic degradation of hydrocarbon compounds.

NEW BIOREMEDIATION PROSPECTS IN ANTARCTICA

The outcomes of applying microbial species for treatment of petroleum hydrocarbon pollution in Antarctica have been tremendous due to the success of great ideas and their effective application in recent decades. Although microbial degradation of petroleum hydrocarbons in Antarctica is well understood nowadays, extra work and effort are needed to optimise and improve the existing approach. Emerging works should be considered and studied to attain the promise they carry. Several developing technologies in microbial bioremediation that show great potential are phytoremediation and the use of genetically-modified bacteria.

According to Greipsson (2011), phytoremediation is the use of plants and associated soil microbes to reduce the concentration or toxic effects of contaminants in the environment. Some advantages of phytoremediation are cost-effective, eco-friendly and generates recyclable energy in ecosystems. Phytoremediation technologies can be categorised as phytostabilisation, phytodegradation, phytovolatilisation and phytoextraction, with comparable results among the systems (Greipsson, 2011). Recent decades have favoured phytoremediation although there are fewer reports on its use in Antarctica (Ali, Khan, & Sajad, 2013; Mitton, Gonzalez, Monserrat, & Miglioranza, 2016).

Successful hydrocarbon remediation through plants has been reported by Jones, Sun, Tang and Robert (2004) and Sun, Lo, Robert, Ray and Tang (2004). Yet, the majority of research with respect to phytoremediation of petroleum hydrocarbons has concentrated on the method of rhizodegradation, due to the cold climate of Antarctica. Rhizodegradation, in general, is defined as the transformation of pollutants in the soil proximal to the roots (rhizosphere) by organisms associated with vegetative species such as bacteria. Though plants and microorganisms can mineralise petroleum hydrocarbons independently of one another, Atlas and Bartha (1998) implied that it is the interaction between plants and microorganisms that is the principal means responsible for petrochemical degradation in phytoremediation efforts. Daryabeigi Zand and Hoveidi (2016) supported the theory of the synergetic energy between plants and microorganisms for the increased degradation effect of recalcitrant compounds based on the positive outcomes in recent studies by Liu, Meng, Tong and Chi (2014a) and Xiao, Liu, Jin and Dai (2015).

In most higher and vascular plants like grasses, several aspects do need to be evaluated for a potential phytoremediator such as their root architecture, exudate patterns of the root, cell wall components and the genetic composition of the plant involved (Balasubramaniyam, 2015). However, certain limitations in vascular plants might occur as plant roots play the most vital part in phytoremediation, specifically rhizodegradation. The presence of hydrocarbons in soil, for instance, may pose a challenge to the growth of plant roots for their toxicity and water stress (Balasubramaniyam, Chapman, & Harvey, 2015), automated hindrance and nutrient deficiency (Brandt, Merkl, Schultze-Kraft, Infante, & Broll, 2006; Merkl, Schultze-Kraft, & Infante, 2005). Meanwhile, studies on phytoremediation using lower plants such as algae, lichens and moss also have increased in recent decades due to their key role in carbon dioxide fixation and immense growth and biomass (Chekroun, Sanchez, & Baghour, 2014; Jacques & McMartin, 2009). While their importance as a potential degrader can be considered as a branch of phytoremediation, most studies on certain lower plants are classified as degradation by microorganisms. In general, the application of phytoremediation in Antarctica might be less practical due to the harsh climate, lack of native plants and soil conditions (Camenzuli, Freidman, Statham, Mumford, & Gore, 2013). Overall, no data have been reported to date on the application of phytoremediation in Antarctic hydrocarbon-polluted sites, except for the mass study by Bramley-Alves, Wasley, King, Powell and Robinson (2014) on the degradation of petroleum hydrocarbons in sub-Antarctic soils by native tussock grass (*Poa foliosa*). Therefore, it is highly encouraged that researchers consider a new branch of subjects regarding this topic.

The idea of employment of genetically-modified (GM) microorganisms (especially bacteria) in hydrocarbon bioremediation has sparked enormous attention among researchers to improve the degradation of hazardous wastes. Past decades have shown several studies on hydrocarbon degradation by GM bacteria such as *Alcaligenes eutrophus* H850 (Van Dyke, Lee, & Trevors, 1996), *Pseudomonas fluorescens* HK44 (Sayler & Ripp, 2000), Sinorhizobium meliloti (Dutta, Hollowell, Hashem, & Kuykendall, 2003), *Pseudomonas putida* PaW 340/pDH5 (Massa et al., 2009) and *Rhodococcus* sp. strain RHA1 (Rodrigues et al., 2001). However, the Antarctic Treaty of 1998 prohibits the introduction of foreign organisms into the Antarctic environment. Hence, GM bacterial species indigenous to the Antarctic are hugely in demand, as no information has been reported on this subject.

CONCLUSION

Recovering and improving Antarctic polluted environments is a real-world concern, as the Antarctic continent can be deemed to be one of the last pristine environments on Earth. In treating pollution ranging from oil spills to soil contamination, bioremediation stands out as an effective approach in the attempt to restore environments to their original conditions. Exploiting the ability of microorganisms to degrade petroleum hydrocarbons, together with the introduction of nutrients, oxygen and other biological means has brought bioremediation to a new level. However, managing this biological means is a meticulous challenge in a harsh environmental condition such as Antarctica.

Improved understanding of the present knowledge such as petroleum hydrocarbon composition and toxicity; management of oil spills; microbial degradation and factors affecting degradation are needed for successful bioremediation. Furthermore, new ideas such as the promising use of phytoremediation (rhizodegradation) and genetically-modified (GM) indigenous bacteria must be looked into for future consideration.

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