UNDERSTANDING THE BEHAVIOUR OF OIL-DEGRADING MICRO-ORGANISMS TO ENHANCE THE MICROBIAL REMEDIATION OF SPILLED PETROLEUM

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Abstract: Petroleum-contamination of both terrestrial and marine environments have persisted as a result of the increasing demand on liquid petroleum globally which has led to the need to clean up spilled petroleum using eco-friendly methods. Of all the petroleum-cleaning techniques explored, the use of petroleum-degrading microbes has received most attention. The microbial remediation of spilled petroleum has been proved to be cost-effective, eco-friendly and sustainable. However, these microbes have been found to thrive under certain environmental/nutritional conditions which influence their behaviour towards spilled petroleum. This study aims to identify the factors responsible for the change in behaviour of oil-degrading microbes which might help facilitate better petroleum spill management. Some of these factors include: the physical nature of the spilled petroleum; chemical nature of the spilled petroleum; availability of nutrients; water temperature; concentration of oxygen; soil region/soil particle size; competition from other micro-organisms. Petroleum-degrading microbes were also found to degrade specific hydrocarbon components in liquid petroleum due to the specific metabolic pathway utilized by individual microbes. This makes the use of a microbial consortium a more aggressive option for the microbial degradation of spilled petroleum than the use of microbial isolates. However, more research on the factors influencing theabundance and productivity of oil-degrading anaerobes may need to be carried out. Also, how oil-degrading microbes can be aided to break down asphalthenes should be investigated.

Keywords: *microbial remediation, biodegradation, oil-degrading microbes, spilled petroleum, oil spillclean-up.*

Introduction

Oil spill incidence and accidents

Every year, an average of about 35 million barrels of petroleum is transported across the seas around the world and this renders the marine ecosystem vulnerable to pollution (Anisuddin et al., 2005). Between 1996 and 1999, an average of 407 oil spills occurred annually in Alaska (Poland et al., 2003). A UNDP (2006) study reports that approximately 273 oil spills have occurred annually in Nigeria between 1976 and 2001. The National Oil Spill Detection and Response Agency (NOSDRA) in Nigeria estimated that the crude oil spilled into the Niger Delta between 1976 and 1996 was more than 2.4 million barrels (The Guardian, 2010), of which 1.82 million (77%) of the total spill was not recovered (Kadafa, 2012). The crude oil was spilled offshore, in swamps and on land (Uyigue and Agho, 2007). Some of the spills were caused by oil pipeline sabotage but the majority of oil spills were due to poor maintenance and monitoring by the oil companies (Aljazeera, 2012). In addition, Egwu (2012) also pointed out that some of the drilling facilities used by the oil companies are out-dated and the procedures employed for preventing oil spills are ineffective.

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The incidence of oil spill accidents is higher on land than on water due to the extremely lengthy (thousands of kilometres long) pipelines which transport crude oil into storage tanks, rail tanks, road tanks and other facilities utilised in the export of liquid petroleum both locally and internationally (Owens, 2000). However, oil spill events on land are confined and often readily containable, while the infrequent oil spill accidents on water are often massive and devastating (Freedman, 1989). This is probably as a result of the greater difficulty in controlling the oil dispersion and spread.

Oil spill incidents are not only caused by pipeline vandalisation and accidents such as collision or explosion of supertankers or oil well blowout. There have been cases of oil spills initiated by natural disasters. For example, in 2005, Hurricane Katrina accounted for the spilling of a total of about 134,300 barrels of crude oil held by Bass Enterprises, Shell, Chevron and Murphy Oil Company in the United States (Llanos, 2005). The deliberate spill of liquid petroleum hydrocarbon into the environment may also occur due to war. Two examples were seen in 1991 during the Gulf war and in 2006 during the Israel-Lebanon crisis, releasing a total of 2-4 million barrels (Khordagui and Al-Ajmi, 1993) and 210,000 barrels (BBC, 2006) of crude oil respectively into the environment. However, the largest and most damaging pollution events usually involve a spill of petroleum from a disabled tanker or drill platform at sea or to a lesser extent from a blowout or a broken pipeline on land (Freedman, 1989).

It is imperative to note that the increasing demand for liquid petroleum may likely not reduce the number of oil spill occurrences. Therefore, oil spill accidents are prone to happen considering the enormous pressure on oil companies/drilling firms to make the petroleum product readily available for global consumption. Whenever there is an oil spill, shorelines, marine waters, groundwater, soils (including farmlands), lakes, rivers and creeks, stand the risk of being severely polluted, and if not controlled within a short time frame, may lead to long-term ecological devastation. Several oil spill remediation techniques for the clean-up of polluted terrestrial and marine environments have been established. However, most of them have been proven to be cost-ineffective and environmentally-unfriendly and hence, unsustainable. As a result, a control measure that will be swift, efficient and sustainable is a necessity. Therefore, attention has been shifted to the use of micro-organisms in cleaning-up both land and water oil spills. Liquid petroleum is composed of numerous hydrocarbon types and it has been observed that individual oil-degrading microbes have special preference for certain hydrocarbon types. Oil-degrading microbes also thrive best under certain environmental/nutritional conditions. This review is aimed at understanding the behaviour of oil-degrading microbes so as to improve the efficacy of microbial remediation of spilled petroleum.

The rate of biodegradation of petroleum hydrocarbonsvariesdepending on the composition and chemical nature of thepetroleum constituents. Crude oil is a liquid petroleum having thousands of hydrocarbon components. Each component has a unique chemical behaviour which makes it eithereasily biodegradable, quite difficult to digest or not degradable at all (The American Academy of Microbiology, 2011).

The chemical composition and structure of Petroleum

Petroleum hydrocarbon molecules can be grouped into four broad categories:

(a) Saturates (branched, unbranched and cyclic alkanes)
(b) Aromatics – ringed hydrocarbon molecules such as monocyclic aromatic hydrocarbons (MAHs) and polycyclic aromatic hydrocarbons (PAHs)

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(c) Resins (polar oil-surface structures dissolved in saturates and aromatics) (Speight, 1999)

(d) Asphalthenes (dark-brown amorphous solids colloidally dispersed in saturates and aromatics) (Balba et al., 1998; Speight, 1999; The American Academy of Microbiology, 2011).

In the structural arrangement of the four main hydrocarbon components of crude oil, saturates make up the outermost layer of the oil whilst asphalthenes constitute the innermost portion of the oil due to their heavier molecular weights.

PAH consist of about sixteen types that are priority pollutants (US EPA, 1993; Othman et al., 2011) but the following three have been the most studied: naphthalene, phenanthrene and pyrene. Alkene, another hydrocarbon component is mainly found in refined petroleum and not in crude petroleum because it is obtained by catalytic cracking (Matar, 1992). Light petroleum hydrocarbons such as gasoline, kerosene and diesel fuel are volatile and easily biodegradable (within days or weeks), so that they rarely persist in marine environments, although they may remain longer in terrestrial environments if buried in sediments, soils, groundwater, or marshes where oxygen levels are very low. However, heavy petroleum hydrocarbons are biodegraded slowly (The American Academy of Microbiology, 2011).Aromatics are more difficult to degrade than alkanes whilst asphalthenes may persist in the environment (The American Academy of Microbiology, 2011).

In general, lighter fractions of petroleum are more soluble in water than heavier ones; aromatics are much heavier than alkanes. Benzene, the lightest MAH has a solubility of 1780g/m^3 whilst naphthalene, the lightest PAH has a solubility of 31g/m^3 (Parker et al., 1971; Clark and MacLoed, 1977). Although alkanes are the most biodegradable petroleum hydrocarbons, those with 5-10 carbon atoms are toxic to most micro-organisms by disrupting their lipid membranes (Bartha, 1986). Furthermore, petroleum hydrocarbons with 20-40 carbon atoms are hydrophobic at room temperature, which probably explains their slow biodegradation (Bartha and Atlas, 1977). Some bacteria, remarkably, produce waxes after degrading crude oil (Ishige et al., 2003).

According to van Hamme et al. (2003),the susceptibility of crude oil components to microbial degradation are in the following order: alkanes >light aromatics (MAHs such as benzene) >cycloalkanes > heavy aromatics (PAHs such as Phenanthrene)> asphalthenes. Resins are easily degraded naturally because they are light polar molecules (Spiecker et al., 2003).

PAHs are made up of more than one benzene ring and those that are made up of two or three cyclic rings such as naphthalene (two-ringed), phenanthrene (three-ringed) and anthracene (three-ringed) with molecular weights of 128g/mol, 178g/mol and 178g/mol respectively, are referred to as low molecular weight or light PAHs (Flores and Mestahoward, 2001). PAHs made up of four rings and above such as pyrene (four-ringed), chrysenes (four-ringed), fluorenthene (five-ringed), benzo [a] pyrene (five-ringed) and coronenes (seven-ringed) with molecular weights of 202g/mol, 228g/mol, 202g/mol, 252g/mol and 300g/mol respectively, are referred to as high molecular weight or heavy PAHs (Niederer et al., 1995; Kanaly and Harayama, 2000; Flores and Mestahoward, 2001; Schulz et al., 2012; MOE, 2013).

PAHs are common petroleum contaminants in the environment considered to be potentially mutagenic and carcinogenic (Boonchan et al., 2000; Mao et al., 2012). BCF (2013) reported that heavy PAHs such as Benzo [a] pyrene damage the DNA of living organisms (i.e. they are genotoxic) and are implicated in human breast cancer. This accounts to a high number of studies on the biodegradation of PAHs in order to safeguard the environment and biodiversity from severe long-term ecological and medical damage by oil spills. However, the focus has been on the biodegradation of light PAHs whilst very little research has been carried out on the biodegradation of heavy PAHs which have been found to be of medical importance.

Asphalthenes are considered to be highly resistant to biodegradation due to their heavy and viscous nature (The American Academy of Microbiology, 2011). Asphalthenes are very complex chemical structures made up of sulphur (0.3 - 10.3%), nitrogen (0.6 - 3.3%), oxygen (0.3 - 4.8%) and trace amounts of metals such as iron, nickel and vanadium (Tavassoli et al., 2012). In addition, asphalthenes have the highest molecular weight of all hydrocarbon compounds in crude oil with values ranging from 600 to $3x10^5$ g/mol and from 1000 to $2x10^6$ g/mol (Speight and Moschopedis, 1981; Kawanaka et al., 1989; Flores and Mestahoward, 2001). This chemical complexity has rendered asphalthenes resistant to microbial attack and unfortunately few studies have been carried out to enhance the potential of biodegradation of asphalthenes.

Factors influencing the microbial remediation of petroleum hydrocarbon

Although it had been established that petroleum hydrocarbons were degraded by oil-eating micro-organisms since the 1940s (Zobell, 1946), the degree to which these processes occurred and the conditions that limited or enhanced biodegradation were not well understood until the 1960s (Le Petit and Barthelemy, 1968).

Oil-degrading microbes are able to break downcrude oil naturally (known as natural attenuation), however, this process is too slow to meet the demands of the environment due to the nutritional imbalance in the ecosystem (Le Petit and Barthelemy, 1968); hence, the need to optimise environmental/nutritional conditions in order to facilitate increasedmicrobial degradation of petroleum hydrocarbon.

Over the years, scientists have manipulated environmental indices in order to understand the factors that encourage microbial metabolism and hydrocarbon degradation, a knowledge which could help restore the environment to a pre-pollution state as early as possible. The main environmental/nutritional factors include the physical nature of the crude oil, the chemical nature of the crude oil, availability of nutrients, concentration of oxygen, water temperature, soil region and soil particle size, and competition from other micro-organisms (Boopathy, 2000; Fountoulakis et al., 2009; The American Academy of Microbiology, 2011).

The physical nature of the spilled petroleum

The physical nature of the spilled petroleum is a factor that affects both terrestrial and marine oil-spill bioremediation. A single large oil slick has a lesser surface area for oil-eating microbes to access compared to numerous small-sized oil slicks. As a result, the physical nature of the hydrocarbon pollutant can either slow down or hasten biodegradation. Also, heavy and viscous hydrocarbon compounds may prove to be recalcitrant as lighter hydrocarbons are quite easier for microbes to digest due to the higher rate of diffusion through the oil-water interface (The American Academy of Microbiology, 2011).

The chemical nature of the spilled petroleum

The chemical nature of the spilled petroleum is determined by the particular hydrocarbon components of the liquid petroleum. Not all hydrocarbon components are biodegradable; some are degradable; some are degraded quickly while others are degraded slowly or not at all. In terrestrial and marine environments, the unbranched alkanes can be degraded within weeks but the branched alkanes and the multiple-ringed aromatic hydrocarbons are resistant to microbial degradation. Asphalthenes, on the other hand, are considered to be the most recalcitrant, thus, could accumulate in the environment (The American Academy of Microbiology, 2011).

Availability of nutrients

Similar to the nutritional needs of other organisms, oil-eating microbes also require nutrients for optimal growth and development. These nutrients are available in the natural environment but occur in low quantities. When there is an oil spill, the petroleum hydrocarbon provides carbon nutrients for the oil-eating bacteria to utilize, but the rate of degradation depends on the availability of other nutrients. The two most limiting nutrients observed are nitrogen and phosphorus which are incorporated into the cellular biomass and stimulate hydrocarbon metabolism (Prince, 1997; McKew et al., 2007a; Calvo et al., 2009). Other nutrients include sulphur and potassium (Evans et al., 2004). These nutrients are limiting factors for both land and water oil spill bioremediation.

Water temperature

The temperature of the surrounding water in which the oil is spilled determines the rate of hydrocarbon degradation whether it is fresh water or marine. Crude oil degradation is faster in warm water because the heat generated within the water body will further encourage the breakdown of the spilled petroleum through natural processes such as evaporation. This leaves the oil-degrading microbes with a smaller size of hydrocarbon pollutant to clean up (The American Academy of Microbiology, 2011). On the other hand, in cold environments such as the Arctic, oil degradation via natural processes is very slow and puts the microbes under more pressure to clean up the spilled petroleum.

The sub-zero temperature of water in this region causes the transport channels within the microbial cells to shut down or may even freeze the entire cytoplasm, thus, rendering most oleophilic microbes metabolically-inactive (Yang et al., 2009). However, some oleophilic microbes are cold-tolerant but are faced with the problem of freeze-thaw seasonal cycle thereby fluctuating between winter and summer, which limits the bioavailability of the spilled petroleum (Yang et al., 2009).

The concentration of oxygen

Oxygen is a gaseous requirement for most living organisms. Similarly, most oleophilic microbes are aerobes (such as *Pseudomonas* and *Proteus*) and a few others are anaerobes (such as *Geobacter*). As a result, environments with low oxygen concentration such as groundwater, deep soils and ocean sediments, have limited rates of hydrocarbon biodegradation compared to the open surfaces where oxygen is readily available for numerous oil-degrading aerobes to digest the pollutants. The presence of oxygen generally accelerates hydrocarbon metabolism (The American Academy of Microbiology, 2011).

The soil region and soil particle size

The soil region and soil particle size are crucial factors limiting the biodegradation of petroleum hydrocarbon in the terrestrial environment. The subsurface soil regions include the vadose zone (unsaturated zone) and the groundwater zone (saturated zone). The surface soil region has the highest microbial population due to the regular input of organic material from plants and animals at the surface whilst the groundwater sediment zone has the lowest microbial population due to the anoxic condition (insufficient oxygen) which increases with soil depth (Adriaens and Hickey, 1993). Many anaerobes and few aerobes have been found to survive in the saturated zone (Boopathy, 2000).

In addition, the size of the soil particle determines the rate of soil permeability, which indirectly affects the rate of petroleum biodegradation. Fine soil particles such as clay have small interstitial spaces which make the soil impermeable, thus, retaining the spilled petroleum at the surface and reducing the bioavailability of microbial nutrients and oxygen. Coarse soil particles such as sand have large interstitial spaces which heavily drain hydrocarbon pollutants through the soil to the unsaturated and saturated zones, oxygen-deficient regions where the rate of biodegradation is very slow.

Moderately drained soils are the optimum requirements for the rapid bioremediation of oil-polluted soils. Soil pH has also been pointed out by the US EPA (2006) to influence bioremediation as it inhibits microbial activity and affects nutrient availability. The generally acceptable soil pH range for optimum bioremediation is 6-8 (US EPA, 2006).

Competition from other micro-organisms

The natural microbial community is composed of oil-degrading microbes that either cooperate together or compete with one another. Competition influences the rate of bioremediation and it could be inter-specific (between members of different species) or intra-specific (between members of the same species). Oil-degrading fungi could compete with oil-eating bacteria not only for the petroleum hydrocarbon but also for the limited nutrients available. As mentioned earlier, some antagonistic oleophilic bacteria have also been found to release metabolites that inhibit the growth and development of other oleophilic bacteria. The interdependence of microbial populations is a requirement for the successful application of bioremediation, which makes competition a limiting factor (The American Academy of Microbiology, 2011).

The appropriate manipulation of the aforementioned environmental and nutritional factors affecting the rate of bioremediation which could be helpful in improving the success of crude oil bioremediation strategies.

Microbial remediation of petroleum hydrocarbons

Microbial remediationhas been argued to be an efficient and sustainable oil spill clean-up technique by many authors (Trindade et al., 2005; Okoh and Trejo-Hernandez, 2006; Tyagi et al., 2011). It is a technology that utilizes the metabolic potential of micro-organisms such as bacteria, fungi and few protozoans (Watanabe, 2001) in degrading liquid petroleum spilled on terrestrial and marine environments, into harmless compounds. These oil-degrading microbes are not living in isolation, hence, referred to as microbial consortia. Of all the known oleophilic micro-organisms, bacteria are the most predominant in the environment (Leahy and Colwell, 1990). These oil-degrading bacteria are sometimes, referred to in literature, as hydrocarbonoclastic bacteria (HCB)

(McKew et al., 2007a). Bacteria's reproductive potential and ubiquity may have made them the most preferred agent of hydrocarbon degradation for bioremediation.

In an oil remediation experiment using oil-utilising bacteria and protozoa as agents of degradation, it was observed that the population of the two agents initially increased which was then followed by a considerable decline in the population of bacteria (Accola, 1994). Acea and Alexander (1988) suggested that the sudden decline in bacterial population may have been "chiefly attributed to predation by protozoa"; although Huang et al. (1981) pointed out that the protozoans may be playing a useful role in stabilising the ecosystem by limiting the possible over-proliferation of bacteria. However, the presence of protozoa tends to disallow the establishment of oleophilic microbial consortia which may explain why not much research has been carried out on protozoa as agents of bioremediation.

The number of micro-organisms capable of utilizing the carbon in crude oil as their sole source of energy is relatively few compared to the general number of microbes available in the environment (Lebkowska et al., 1995). This is the reason it is important to have a good understanding of the available oil-degrading micro-organisms found both on land and water. Oil-eating micro-organisms produce enzymes which break down hydrocarbon compounds through single or multiple metabolic pathways.

The enzymes produced are incapable of breaking down all forms of hydrocarbon compounds; as a result, most oleophilic microbes are hydrocarbon-specific, although a few are physiologically-versatile and can degrade a wide-range of hydrocarbons (see *Table 1*). For example, toluene, an aromatic hydrocarbon, can be broken down via multiple metabolic pathways by the enzyme, toluene 2-monoxygenase (Ramos et al., 2011), produced by a toluene-degrading microbe (such as *Rhodococcus* spp.). The metabolic pathways include five aerobic pathways and at least one anaerobic pathway (Pazos et al., 2004). This biochemical diversity and hydrocarbon-specificity displayed by oleophilic microbes, if expressed in synergy by microbial consortia, will enhance bioremediation (Roling et al., 2002).

Table 1*.The habitat and hydrocarbon-specificity of40 oleophilic micro-organisms*

Oleophilic micro-organisms	**Bacteria/ Fungi**	**Habitat**	**Hydrocarbon-specificity**
Pseudomonas spp. (Kanaly and Harayama, 2000)	Bacterium	Soil, river and marine	Benzene, toluene, ethylbenzene, xylene, naphthalene, phenanthrene, kerosene and diesel (Watanabe, 2001; Joshi and Pandey, 2011)
Cycloclasticus spp.	Bacterium	Marine	PAH (not specific) (Watanabe, 2001)
Alcanivorax spp. (Watanabe, 2001)	Bacterium	Soil, river and marine	Alkanes (Yakimov et al., 1998; Harayama et al., 1999; Chang et al., 2000)

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Geobacter spp.	Bacterium	Groundwater, deep soil and ocean sediments	Anaerobic breakdown of benzene (Watanabe, 2001)
Syntrophus spp.	Bacterium	Groundwater, deep soil and ocean sediments	Anaerobic breakdown of hexadecane (Zengler et al., 1999)
Burkholderias spp.	Bacterium	Soil	PAH (not specific) (Watanabe, 2001) and xylene (Bacosa et al., 2012)
Sphingomonas spp.	Bacterium	Soil	Pyrene (Ho et al., 2000)
Mycobacterium spp.	Bacterium	Soil	Benzo [a] pyrene and pyrene (Watanabe, 2001)
Rhodococcus spp. (de Carvalho et al., 2005)	Bacterium	Soil, river and marine	Benzene, toluene, ethylbenzene, xylene, anthracene and Benzo [a] pyrene (Farhadian et al., 2008)
Ralstonia spp. (de Carvalho et al., 2005)	Bacterium	Soil, river and marine	Benzene, toluene, ethylbenzene and xylene (Farhadian et al., 2008)
Haemophilus spp.	Bacterium	Soil, river and marine	Phenanthrene and pyrene (McKew et al., 2007a)
Thalassolituus oleivorans	Bacterium	Marine	Phenanthrene and pyrene (McKew et al., 2007a)
Proteus spp.	Bacterium	Soil	Xylene and diesel (Joshi and Pandey, 2011)
Bacillus spp.	Bacterium	Soil	Toluene and diesel (Joshi and Pandey, 2011)
Mesorhizobium spp.	Bacterium	Soil	Most PAH (not specific) (Mao et al., 2012)
Alcaligenes spp.	Bacterium	Soil	Most PAH (not specific) (Mao et al., 2012)
Nocardia nova	Bacterium	Soil	A wide spectrum of petroleum hydrocarbon (Trindade,2002)

Rhodotorula glutinis var. dairenesis	Fungus	Soil	A wide spectrum of petroleum hydrocarbon (Trindade,2002)
Planococcus spp.	Bacterium	Soil	Light Arabian oil (Engelhardt et al., 2001; Evans et al., 2004)
Stenotrophomonas spp.	Bacterium	Soil	PAH (not specific) (Vinas et al., 2002; Vinas et al., 2005)
Ochrobactrum spp.	Bacterium	Soil	PAH (not specific) (Vinas et al., 2002; Vinas et al., 2005)
Pandorea spp.	Bacterium	Soil	PAH (not specific) (Vinas et al., 2002; Vinas et al., 2005)
Labrys spp.	Bacterium	Soil	PAH (not specific) (Vinas et al., 2002; Vinas et al., 2005)
Fusarium spp.	Fungus	Soil	PAH (not specific) (Vinas et al., 2002; Vinas et al., 2005)
Fundibacter spp.	Bacterium	Soil, river and marine	Alkanes (not specific) (Bruns and Berthe-Corti, 1999)
Pseudomonas paucimobilis	Bacterium	Soil, river and marine	Fluoranthene (Mueller et al., 1990)
Micrococcus spp.	Bacterium	Soil/sludge	Low molecular weight PAH (Othman et al., 2011)
Corynebacterium spp.	Bacterium	Soil/sludge	Low molecular weight PAH (Othman et al., 2011)
Pediococcus spp.	Bacterium	Soil/sludge	Low molecular weight PAH (Othman et al., 2011)
Sphingobacterium spp	Bacterium	Soil/sludge	Low molecular weight PAH (Othman et al., 2011)
Tsukamurella spp.	Bacterium	Soil/sludge	Low molecular weight PAH (Othman et al., 2011)
Phanerochaete spp.	Fungus	Soil	PAH (not specific) (Li et al., 2009)
Chrysosporium spp.	Fungus	Soil	PAH (not specific) (Li et al., 2009)
Cuuninghamella spp.	Fungus	Soil	PAH (not specific) (Li et al., 2009)
Alternaria alternate	Fungus	Soil	PAH (not specific) (Li et al., 2009)
Penicillium chrysogenum	Fungus	Soil	PAH (not specific) (Li et al., 2009)
Aspergillus niger	Fungus	Soil	PAH (not specific) (Li et al., 2009)
Zoogloea spp.	Bacterium	Soil	PAH (not specific) (Li et al., 2009)

Flavobacterium spp.	Bacterium	Soil	PAH (not specific) (Li et al., 2009)
Cupriavidus spp.	Bacterium	Soil; marine	Decane (Bacosa et al., 2012)

Besides the catabolic potential of oil-degrading microbes, other strategies have been adopted by these microbes to enhance the detoxification of pollutants, such as the modification of their cell membrane to preserve their structural integrity when in contact with pollutants (de Carvalho et al., 2005); the production of surface active compounds known as biosurfactants which helps to partially weather the pollutant, thereby, rendering it vulnerable to microbial degradation (Ron and Rosenberg, 2002) and the release of toxic pollutants from within the microbe’s protoplasm via efflux pumps, active transporters located in the cytoplasmic membrane (van Hamme et al., 2003).

Competition amongst oil-degrading micro-organisms has also been observed. Some oleophilic microbial consortia develop synergistic relationships whilst few others show competitive relationships. For example, *Cycloclasticus* and *Alcanivorax borkumensis* show a synergistic association because *A. borkumensis* produces an extracellular lipid biosurfactant which render PAHs readily available for digestion by *Cycloclasticus* and the competition for hydrocarbon resources is absent because *A. borkumensis* feeds on another hydrocarbon (alkanes) (Yakimov et al., 1998; McKew et al., 2007a). However, *Thalassolituus* exhibits an antagonistic relationship with other oil-eating bacteria by producing a metabolite which inhibits their growth and development (Yakimov et al., 2005).

It has been well established that the presence of microbial consortia rather than individual microbes will enhance bioremediation; therefore, the antagonistic potential of some oil-degrading microbes could limit the efficiency of microbial remediation. The activities of these antagonistic oil-degrading bacteria have to be controlled in order to encourage the continued development of viable oil-degrading microbial consortia.

Conclusion

The behaviour of oil-degrading micro-organisms towards spilled petroleum has beeninvestigated. Oil-degrading microbes modify their cell membranes, produce biosurfactants and eject toxic pollutants from their protoplasm in order to survive petroleum-contaminated areas. The hydrocarbon-specificity displayed by oil-degrading micro-organisms is as a result of the metabolic pathway individually utilized. Microbes capable of utilizing multiple biochemical pathways find it easy to degrade a wider range of hydrocarbon compounds.Of all the four basic components of petroleum, asphalthene is the most recalcitrant to microbial degradation due to its chemical complexity. Also, it appears that there are more oil-degrading aerobes than anaerobes, and most of these aerobes are terrestrial in habit. The study of the behaviour and factors influencing the microbial metabolism of hydrocarbon compounds will ultimately aid the understanding of how best these factors and elements can be manipulated to achieve an accelerated microbial degradation of spilled petroleum world-wide.

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