

Bioremediation of Petroleum Contaminants in Aquatic Environments (Review Article)

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Abstract: Oil spill incidents are of particular concern in the environment. Petroleum and its components are a considerable threat to the environment and to human health. Mechanical and chemical methods generally used to remove oil contaminants from contaminated sites have limited effectiveness and can be expensive. Bioremediation is a potentially important option for dealing with the oil spills in the ecosystem. Bioremediation is the promising technology for the treatment of these contaminated sites since it is cost-effective, eco-friendly method and will lead to complete mineralization of organic contaminants into carbon dioxide, water, inorganic compounds, etc... and transformation of complex organic contaminants to other simpler organic compounds by biological agents like microorganisms. Many microorganisms in water are capable of degrading oil contaminants including hydrocarbons. However, these microorganisms have been found to thrive under certain environmental/nutritional conditions which influence their behaviour towards spilled petroleum. Some of these factors include: the physical and chemical nature of the spilled petroleum; availability of nutrients; water temperature; concentration of oxygen; etc... This review paper presents an overview of petroleum degradation by microorganisms and some other recent techniques in the aquatic environments.

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Introduction

Water contaminated with petroleum oil and its components especially hydrocarbons is common in the oil-producing and industrialized countries of the world and is a considerable threat to the environment and to human health (Al-Baldawi *et al.*, 2015).

Petroleum and its components drive the present civilization and are the major energy sources for industry and daily life. But, where there is use there is a chance for abuse too. Hence, being the prime source of energy, petroleum is also a major environment pollutant (Phulia *et al.*, 2013; Zaki *et al.*, 2013).

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; Macaulay, 2015). The oil spill accidents on water are often massive and devastating (Freedman, 1989). During the period 1990–2001, more than 200 ship accidents were occurred and some of those resulted in major spills into the marine environment (Zaki *et al.*, 2014). This is probably as a result of the greater difficulty in controlling the oil dispersion and spread (Macaulay, 2015). However, accidents are not the sole cause of petroleum contamination scenarios. Exploration, transportation, refining, storage and consumption of petroleum and petroleum products

cause a continuous release of hydrocarbons into previously pristine areas through improper disposal or leakage in storage systems (Head and Swannell, 1999; Phulia *et al.*, 2013; Montagnoli *et al.*, 2015).

Oil spill incidents are not only caused by pipeline vandalism and accidents such as collision or explosion of supertankers or oil well blowout. There have been cases of oil spills initiated by natural disasters as Hurricanes and due to wars. 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; Macaulay, 2015). The amount of natural crude oil seepage was estimated to be 600,000 metric tons per year with a range of uncertainty of 200,000 metric tons per year (Kvenvolden and Cooper, 2003; Das and Chandran, 2011). Since 1992, there have been 21 major oil spills causing huge economic and immeasurable non-economic losses (Zaki *et al.*, 2013).

The sudden input of large amounts of petroleum hydrocarbons, as occurs with spillages, stresses the environment in a way not imposed by natural hydrocarbon inputs. The ecologic effects of oil spillages generally depend upon the location of the spill, the type of oil spilled, and the amount of oil spilled. Catastrophic oil spillages often occur in

nearshore regions, affecting both marine and coastal ecosystems. Such spillages often involve a relatively large amount of oil within a small region. The eventual fate and ecologic effects of oil spills, large or small, depend upon many factors (**Bartha and Atlas, 1977**).

Petroleum contamination is quite harmful for the higher organisms (**Lyons et al., 1999; Janjua et al., 2006; Cheong et al., 2011**) but it is fortunate that microorganisms can thrive on it and assimilate (**Atlas, 1995; de Oliveira et al., 2012**). Soon after major oil spill incident is reported, the efforts are concentrated at physical collection of the spilled oil with skimmers is the first response but this is rarely completely successful (**Prince, 1993**). As per Office of Technology Assessment (OTA; USA), such mechanical methods are efficient at removing no more than 10-15 per cent of oil after a major spill (**Phulia et al., 2013**).

Crude oil is perhaps the most complex mixture of organic compounds that occurs on Earth. Recent advances in ultra-high-resolution mass spectrometry have allowed the identification of more than 17,000 distinct chemical components, and the term petroleomics has been coined to express this newly uncovered complexity (**Marshall and Rodgers, 2004; Head et al., 2006**). Crude oil is a mixture of many compounds such as alkanes, aromatics, resins and asphaltenes (**Plaza et al., 2008**). In general, lighter fractions of petroleum are more soluble in water than heavier ones; aromatics are much heavier than alkanes. Benzene, the lightest monocyclic aromatic hydrocarbons (MAHs), has a solubility of 1780g/m³ whilst naphthalene, the lightest polycyclic aromatic hydrocarbons (PAHs), has a solubility 31g/m³ (**Parker et al., 1971; Clark and MacLeod, 1977**) and toxic to aquatic life.

Polycyclic aromatic hydrocarbons (PAHs) have been known to affect a variety of biological processes and may exhibit a wide range of hazardous effects to aquatic organisms including acute toxicity, developmental and reproductive toxicity, photo-induced toxicity, and can be potent cell mutagens and carcinogens (**Gaspere et al., 2009; Karacik et al., 2009**), in addition to well documented sublethal effects which include morphological and histopathological damage (**Brown et al., 1996; Carls et al., 1999; Heintz et al., 1999**), physiological and stress effects, endocrine disruption (**Kennedy and Farrell, 2006**), and ecological effects (**Reddy et al., 2002**). Sediment-associated PAHs are known to exhibit narcotic effects in benthic organisms (**Di Toro and McGrath, 2000**) but also have been implicated in the development of tumors in bottom feeding fish (**Myers et al., 1991; Balch et al., 1995**) and in the induction malformation, loss of fertility or immune

deficiency in aquatic vertebrates (**Reynaud et al., 2004**) and invertebrates (**Gagnaire et al., 2006**) including oysters (**Jeong and Cho, 2005**). Due to their lipophilicity, PAHs are known to accumulate in sediments as well as in fish and shell fish especially in mussels and other aquatic invertebrates (**Gewurtz et al., 2000**). By accumulating in invertebrates, PAHs also enter the aquatic food webs and also pose a risk to human health via consumption of seafood (**Okay et al., 2003; Karacik et al., 2009**). It has been reported that consumption of shellfish polluted by PAHs may cause lung cancer in humans (**Law and Klungsoyr, 2000; Gaspere et al., 2009; Zaki et al., 2014**).

Although alkanes are the most biodegradable petroleum hydrocarbons, those with 5-10 carbon atoms are toxic to most microorganisms 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**). 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)> asphaltenes (**Macaulay, 2015**). Resins are easily degraded naturally because they are light polar molecules (**Spiecker et al., 2003**).

Many microorganisms possess the enzymatic capability to degrade petroleum hydrocarbons. Some microorganisms degrade alkanes (normal, branched and cyclic paraffins), others aromatics, and others both paraffinic and aromatic hydrocarbons (**Atlas, 1981; Leahy and Colwell, 1990; Atlas and Bartha, 1992; Atlas, 1995**). In such cases, bioremediation has a major role to play in neutralizing the harmful effects of oil in the open environment. The basic principle is to use organisms that can use petroleum as carbon source and hence, break them down to harmless end products (**Phulia et al., 2013**).

Bioremediation of Petroleum Contaminants

Mechanical removal of contaminants (e.g. hydrocarbons) from the environment relies on expensive, slow, and inefficient methodologies (**Mandri and Lin 2007**). Biological treatment, or bioremediation, is an environmental-friendly strategy gaining increasing prominence for its potential to clean up oil contaminated marine ecosystem (**Head and Swannell, 1999; Head et al., 2006; Beolchini et al., 2010; Dell'Anno et al., 2012**). In addition, bioremediation technology is a desirable alternative and believed to be noninvasive, relatively cost-effective and important method for the treatment of oil-contaminated sites (**April et al., 2000; Zhu et al., 2004; Karhu et al., 2009; Montagnolli et al., 2015**). Bioremediation can be defined as an engineered

process that uses microorganisms to destroy, degrade, detoxify or remove environmental hazardous pollutants owing to their diverse metabolic capabilities is an evolving method for the removal and degradation of many environmental pollutants including the products of petroleum industry (**Medina-Bellver et al., 2005**) and transform them into less complex and harmless end products such as carbon dioxide and water (**Coulon et al., 2010; Malik and Ahmed, 2012; Alwan et al., 2013; Gomez and Sartaj, 2014**).

For bioremediation to be successful, the bioremediation methods depend on having the right microbes in the right place with the right environmental factors for degradation to occur. The right microbes are bacteria or fungi, which have the physiological and metabolic capabilities to degrade the pollutants (**Boopathy, 2000**). Like any other technology that uses biological agents, success of bioremediation of petroleum contamination depends on establishing and maintaining conditions that favor proliferation of petroleum scavenging microorganisms (**Das and Chandran, 2011**). Numerous scientific review articles have covered various factors that influence the rate of oil biodegradation (**Zobell, 1946; Atlas, 1981; Foght and Westlake, 1987; Leahy and Colwell, 1990; Atlas, 1992; Atlas and Bartha, 1992; Das and Chandran, 2011**).

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 (**Macaulay, 2015**).

Requirements of a Successful Bioremediation Process of Petroleum Contaminants

1. Availability of microorganisms

The very first requirement is the availability of microorganisms, with the appropriate metabolic capabilities, that can utilize oil as a metabolic substrate. Finding and transplanting such an organism to the site of contamination would be the first approach (**Zaki et al., 2013**). **Jones et al. (1983)** reported for the first time biodegraded petroleum byproducts in marine sediments (**Das and Chandran, 2011**).

If these microorganisms are present, then optimal rates of growth and hydrocarbon biodegradation can be sustained by ensuring that adequate concentrations of nutrients and oxygen are present and that the pH is between 6 and 9. The physical and chemical characteristics of the oil and oil surface area are also important determinants of bioremediation success (**Das and Chandran, 2011**). Bioaugmentation and Biostimulation are the two main approaches followed in this regard. Bioaugmentation refers to inoculating the affected area with degrading

microorganisms (laboratory-grown bacteria that have appropriate degradative abilities) while biostimulation would require favoring growth of such microorganism through addition of nutrients or by providing other growth-limiting substrates (e.g. oxygen, surf washing, electron acceptors, or electron donors etc.) (**Inoue et al., 2005; Venosa and Zhu, 2005**). As petroleum is hydrophobic in nature, its bioavailability becomes a major constraint in the process of bioremediation. Use of biosurfactants is a common approach to increase the bioavailability (**Phulia et al., 2013**).

Different organisms have varied degradation capabilities and act on different substrates. As petroleum is an assortment of different components, it is advisable to use a cocktail of organisms to effect remediation (**Zaki et al., 2013; Phulia et al., 2013**). Enzymatic degradation of petroleum can be achieved by bacteria, algae or fungi. **Floodgate (1984)** mentioned 25 genera of hydrocarbon degrading bacteria and 25 genera of hydrocarbon degrading fungi which were isolated from marine environment.

Bacteria are the most efficient of all organisms that can degrade hydrocarbons (**Rahman et al., 2003; Brooijmans et al., 2009**). Biostimulation strategies applied to accelerate the abatement of hydrocarbon contamination are expected to be associated with changes in bacterial biodiversity and assemblage composition (**Widdel and Rabus, 2001; Head et al., 2006; Dell'Anno et al., 2012**).

Several bacteria are even known to feed exclusively on hydrocarbons (**Yakimov et al., 2007**) in aquatic environments. They tolerate high concentrations of the hydrocarbons and have a high capability for their degradation. Most of them belong to *Acinetobacter* spp., *Acinetobacter Iwoffii*, *Alcaligenes* spp., *Arthobacter*, *Bacillus* spp., *Bacillus subtilis*, *Brevibacterium*, *Burkholderia*, *Corynebacterium* spp., *Flavobacterium* spp., *Alcanivorax* spp., *Micrococcus roseus*, *Mycobacterium*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Rhodococcus*, *Sphingomonas*, *Achromobacter*, *Nocardia*, *Vibrio*, *Brevibacterium*, *Corynebacterium*, and *Xantomonas* species (**Bartha and Atlas, 1977; Jones et al., 1983; Adebuseye et al., 2007; Nadarajah et al., 2002; Okoh and Trejo-Hernandez, 2006; Jacques et al., 2008; Onbasili et al., 2011**). In the past 10 years, several interesting marine bacteria that are specialists adapted to hydrocarbon degradation have been isolated (**Head et al., 2006**). These bacteria use hydrocarbons almost exclusively as a carbon source, and they include *Alcanivorax* spp. (**Yakimov et al., 1998**), *Cycloclasticus* spp. (**Dyksterhouse et al., 1995**), *Oleiphilus* spp. (**Golyshin et al., 2002**), *Oleispira* spp. (**Yakimov et al., 2003**), *Thalassolituus* spp. (**Yakimov et al., 2004**) and some members of the

genus *Planomicrobium* (previously known as *Planococcus*) (Engelhardt *et al.*, 2001). *Alcanivorax* spp., *Oleiphilus* spp., *Oleispira* spp. And *Thalassolituus* spp. use a variety of branched- and/ or straight-chain saturated hydrocarbons, as does *Planomicrobium alkanoclasticum* MAE2 (previously known as *Planococcus alkanoclasticus* MAE2). By contrast, *Cycloclasticus* spp. have evolved to use a range of polycyclic aromatic hydrocarbons (Head *et al.*, 2006). Some bacteria, remarkably, produce waxes after degrading crude oil (Ishige *et al.*, 2003).

Some fungal and yeast genera utilized for this purpose are *Amorphoteca*, *Neosartorya*, *Talaromyces*, *Graphium*, *Candida lipolytica*, *Yarrowia*, *Pichia*, *Aspergillus*, *Cephalosporium*, *Spurobolomyces*, *Cladosporium resinae*, *Rhodotorula mucilaginosa*, *Geotrichum* spp., *Trichosporon mucoides* and *Penicillium* (Bartha and Atlas, 1977; Boguslowska-Wąs and Dąbrowski, 2001; Chaillan *et al.*, 2004; Singh, 2006). After the potential scavengers have been identified, the conditions for their survival and proliferation have to be ascertained.

Though algae and protozoa are the important members of the microbial community in aquatic ecosystems, reports are scanty regarding their involvement in hydrocarbon biodegradation. Walker *et al.* (1975) isolated an alga, *Prototheca zopfi* which was capable of utilizing crude oil and a mixed hydrocarbon substrate and exhibited extensive degradation of n-alkanes and isoalkanes as well as aromatic hydrocarbons. The achlorophyllous algae *Prototheca hydrocarbonnea* was reported to degrade hydrocarbons (Bartha and Atlas, 1977). *Scenedesmus* strains utilized n-heptadecane mixotrophically, but were unable to grow on this substrate in the dark (Masters and Zajic, 1971). Cerniglia *et al.* (1980) observed that, nine cyanobacteria, five green algae, one red alga, one brown alga, and two diatoms could oxidize naphthalene. Protozoa, by contrast, had not been shown to utilize hydrocarbons (Das and Chandran, 2011). In addition, many scientists reported that, in the case of algae and protozoa, the evidence suggests there is no ecologically significant role played by these groups in the degradation of hydrocarbons (Bossert and Bartha, 1984; O'Brien and Dixon, 1976; Saadoun and Al-Ghzawi, 2005).

2. Factors influencing the microbial bioremediation of petroleum hydrocarbon

A number of limiting factors have been recognized to affect the biodegradation of petroleum hydrocarbons (Das and Chandran, 2011). 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, pH and water temperature (Boopathy, 2000; Fountoulakis *et al.*, 2009; The American Academy of Microbiology, 2011; Macaulay, 2015).

A. The physical nature of the spilled petroleum

The physical nature of the spilled petroleum is a factor that affects marine oil-spill bioremediation. Crude oil in aquatic systems, usually does not mix with seawater, and therefore, floats on the surface, allowing the volatilization of the 12 carbons or less components. The rate of dispersion of the floating oil will depend on the action of waves which in turn is dependent on the weather (Saadoun and Al-Ghzawi, 2005). 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; Macaulay, 2015).

B. 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 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. Singer and Finnerty (1984) mentioned that alkanes are usually the easiest hydrocarbons to be degraded by their conversion to alcohol via mixed function oxygenase activity. Asphaltenes, on the other hand, are considered to be the most recalcitrant, thus, could accumulate in the environment (The American Academy of Microbiology, 2011; Macaulay, 2015). In addition, the simpler aliphatics and monocyclic aromatics are readily degradable, but more complex compounds such as PAHs are not easily degraded and may persist for some time (Saadoun and Al-Ghzawi, 2005).

C. 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 (Macaulay, 2015). 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. Biodegradation in aquatic environment is limited by the availability of nutrients such as

nitrogen, phosphorous, and in some cases iron which are necessary for initial microbial cell growth (Cooney, 1984; Prince, 1997; McKew *et al.*, 2007; Calvo *et al.*, 2009; Thavasi *et al.*, 2011) and stimulate hydrocarbon metabolism. Other nutrients include sulphur and potassium (Evans *et al.*, 2004). It is well known that nitrogen and phosphorus are the major nutrients for productivity in an aquatic habitat (Phulia *et al.*, 2013). At the time of oil spills, there is a sudden increase in the carbon and hence, nitrogen and phosphorus would become major rate limiting factors (Cooney *et al.*, 1985). A sudden increase in the carbon:nitrogen ratio or carbon:phosphorus ratio at the time of oil spill or contamination would restrict the growth of microbes (Leahy and Colwell, 1990) hence, it would be vital to restore the balance by adding oleophilic fertilizers. On the other hand, excessive nutrient concentrations can also inhibit the biodegradation activity (Chaillan *et al.*, 2006). Several authors have reported the negative effects of high nutrient levels on the biodegradation of hydrocarbons (Oudot *et al.*, 1998; Châneau *et al.*, 2005) especially on aromatics (Carmichael and Pfaender, 1997). The optimum nutrient balance required for hydrocarbon remediation is Carbon: Nitrogen: Phosphorus equals 100:10:4 (Thapa *et al.*, 2012). However, in the regions where there is high nitrogen background, nutrition addition has no or rather negative effect on bioremediation rates and hence, fertilization is recommended only for those regions that are deficient in nutrients (Oudot *et al.*, 1998; Phulia *et al.*, 2013).

D. Water temperature

Among the physical factors temperature is most important one determining the survival of microorganisms and composition of the hydrocarbons (Das and Chandran, 2011). In addition, temperature plays a significant role in controlling the nature and the extent of microbial hydrocarbon metabolism, which is of special significance for in situ bioremediation (Margesin and Schinner, 2001). The temperature of the surrounding water in which the oil is spilled determines the rate of hydrocarbon degradation whether it is freshwater 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 (Macaulay, 2015). 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; Macaulay, 2015). 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; Macaulay, 2015).

For freshwater bioremediation process 20-30 °C is the ideal temperature while for marine 15-20 °C is recommended. For high molecular weight polycyclic hydrocarbons, which are otherwise difficult to degrade, higher temperatures may be required (Bartha and Bossert, 1984; Cooney, 1984; Phulia *et al.*, 2013). At low temperatures, the rate of biodegradation of oil is reduced as a result of the decreased rate of enzymatic activities, or the "Q₁₀" (the change in enzyme activity caused by a 10 °C rise) effect (Atlas and Bartha, 1972; Gibbs *et al.*, 1975), hence, higher temperature would favor bioremediation. It was reported that the rate of hydrocarbon remediation was maximum in the range of 30-40 °C in general and above this, the membrane toxicity effect of hydrocarbons was found to inhibit the survival of microorganisms (Bartha and Bossert, 1984). As there is a close relationship between temperature and oil bioremediation, it is easy to understand why an oil leak disaster would be dangerous in Polar Regions (Phulia *et al.*, 2013; Zaki *et al.*, 2013). Temperate zone lakes have in general more extreme water temperature variations than the oceans in the same climatic region (Bartha and Atlas, 1977). Ward and Brock (1976) studying limiting factors of oil biodegradation in temperate lakes of Wisconsin found water temperature to be the predominant limiting factor throughout the fall, winter, and spring, and only during the summer did nutrient limitation take precedence.

E. The concentration of oxygen

Oxygen is a gaseous requirement for most living organisms. In most petroleum-contaminated sediments and water, oxygen usually is the limiting requirement for hydrocarbon biodegradation (Hinchee and Ong, 1992; Miller *et al.*, 1991) because the bioremediation methods for reclamation of these contaminated sites is mainly based on aerobic processes (Saadoun and Al-Ghazawi, 2005). 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 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; Macaulay, 2015**).

The first step in degrading hydrocarbons is action of oxygenase which requires molecular oxygen (**Das and Chandran, 2011**). Bacteria and fungi in their breaking down of aliphatic, cyclic and aromatic hydrocarbons involve oxygenase enzymes (**Singer and Finnerty, 1984; Perry, 1984; Cerniglia, 1984**), for which molecular oxygen is required (**Atlas, 1984**). By the action of monooxygenases (on aliphatic and certain aromatic hydrocarbons) or dioxygenases (on aromatic hydrocarbons), one or two oxygen atoms, respectively, are directly incorporated from O₂ leading to hydroxylated products (**Widdel and Rabus, 2001**). As hydrocarbons are less denser than water, they would form a layer over water and oxygen limitation would rarely be encountered. But, under the condition of large spill and extensive bacterial colonies thriving on it, oxygen may get depleted soon leading to anaerobic conditions (**Thapa et al., 2012**). Oxygen though usually not a rate determining step may become limiting when aquatic sediments are deeper or water movement through small pores of sediment is restricted so that oxygen is not replenished (**Salleh et al., 2003**). Though contribution of anaerobic bacteria to bioremediation is still considered negligible (**Phulia et al., 2013**). **Widdel and Rabus (2001)** report that some anaerobic bacteria may still have some role to play while it is a matter of time till they are discovered. Some anaerobic bacteria that may have role to play in hydrocarbon degradation are *Syntrophus* spp., *Methanosaeta* spp., *Metanospirillum* spp., *Desulfotomaculum* spp., *Geobacter* spp. (**Watanabe, 2001; Phulia et al., 2013**).

F. pH

pH had an implication on biodegradation rates. The rates were found to be highest at neutral pH (**Leahy and Colwell, 1990**). Lower pH at around 5.0 (**Patrick Jr and DeLaune, 1977**) as seen in salt marshes reduces oil mineralization but the rates were satisfactory at pH above 6.5 (**Hambrick III et al., 1980**). Octadecane mineralization improved further at pH 8.0 (**Leahy and Colwell, 1990**). The overall biodegradation rate of hydrocarbons is generally higher under slightly alkaline conditions (**Saadoun and Al-Ghzawi, 2005**).

G. Salinity

Microbial communities have salinity tolerance and this aspect is important for aquatic ecosystems as salinity is very much relevant when bioremediation of water comes into discussion. While freshwater and estuarine communities can survive in sea water, the inverse is not successful (**Salleh et al., 2003**). As sea water is buffered and salinity is constant in deeper regions maintenance is required only in

expanses where freshwater dilutions may vary with time. Salinity maintenance is required for the inland regions where salinities may change with surface runoff. In sea water, the autochthonous communities degrade hydrocarbons efficiently and the degradation can be significant till the salinities are in the range of 0.1 to 2.0 M NaCl, 0.4 M NaCl being the salinity level of natural sea water (**Salleh et al., 2003**). **Ward and Brock (1978)** established a negative relation between salinity and biodegradation of petroleum contaminants owing to less survival of microbial communities in very high salinities (33-284 g/L). Also, **Díaz et al. (2000)** found that the biodegradation of crude oil was greatest at lower salinities and decreased at salinities more than twice that of normal seawater. However, **Shiaris (1989)** reported a general positive correlation between salinity and rates of mineralization of phenanthrene and naphthalene in estuarine sediments. In another study, **Mille et al. (1991)** noted that the amount of oil degraded initially increased as the salt concentration increased to a level of 0.4 mol/L (23.3 g/L) of NaCl and thereafter decreased with increasing salt concentration. However, some bacteria like *Streptomyces albaxialis* (**Kuznetsov et al., 1992**) for crude oil degradation and *Halobacterium* spp. (**Kulichevskaya et al., 1992**) for degradation of n-alkanes (C₁₀-C₃₀) have been identified. **Kapley et al. (1999)** cloned *E. coli* pro U operon, which is responsible for osmoregulation, into some bacterial consortium which can attack various fractions of crude oil making them salinity tolerant upto 6 per cent NaCl (**Zaki et al., 2013**).

H. Pressure

The importance of pressure is confined to the deep-ocean environment where the oil that reaches there will be degraded very slowly by microbial populations (**Saadoun and Al-Ghzawi, 2005**). Thus, certain recalcitrant fractions of the oil could persist for decades (**Colwell and Walker, 1977**). **Schwarz et al. (1974a,b and 1975)** monitored the degradation of hydrocarbons by a mixed culture of deep-sea sediment bacteria under 1 atm and 495 or 500 atm at 4 °C. After 40-week high pressure incubation, 94% of the hexadecane was degraded, the same amount that occurred after 8 weeks at 1 atm (**Schwarz et al., 1975**).

3. Biosurfactants

Bioavailability of petroleum is a major problem that limits the rate of biodegradation. In order to enhance bioavailability, it is must that solubilization be increased. Such a task is accomplished by certain microorganisms that secrete biosurfactants which are a group of surface active chemicals that increase the bioavailability of petroleum floating on the water column by increasing their solubilisation (**Das and Chandran, 2011**). Biosurfactants are structurally

diverse amphipathic surface-active compounds produced by a wide array of microorganism genera. As compared to their synthetic counterparts, biosurfactants are biodegradable, environmentally safe, stable under extreme conditions, and they can be produced in situ from inexpensive renewable substrates (Desai and Banat, 1997; Benincasa *et al.*, 2010). Low toxicity and the high biodegradability make biosurfactants advantageous when compared to synthetic compounds. These are desirable properties to bioremediation strategies and processes (Montagnoli *et al.*, 2015).

Biosurfactants increase the oil surface area and hence, the amount of oil that is actually available for degradation to the bacteria. Due to this property of enhancing biodegradation of oil such surfactant producing bacteria have potential to be used in bioremediation (Cameotra and Singh, 2008). A consortium of bacteria was used for evaluation of surfactants and their composition by Cameotra and Singh (2008). The surfactant was found to be a conglomerate of 11 rhamnolipid family members and found that crude biosurfactant addition to the oil contamination was very effective in degradation process. Genus *Pseudomonas* is widely known for efficient surfactant production properties (Beal and Betts, 2000; Rahman *et al.*, 2007; Cameotra and Singh, 2008; Pornsunthorntaweew *et al.*, 2008; Phulia *et al.*, 2013).

4. Phytoremediation

Phytoremediation is a broad term that has been in use since 1991 that uses plants and their associated microorganisms to recover water and to manage a wide variety of environmental pollution problems, including the cleanup and reduce the volume, mobility, or toxicity of contaminants in effected media contaminated with hydrocarbons and other hazardous substances (Das and Chandran, 2011; Alwan *et al.*, 2013). It is more environmentally friendly than the conventional mechanical clean-up methods (Barrutia *et al.*, 2011; Liu *et al.*, 2011; Al-Baldawi *et al.*, 2015).

It is an emerging green technology that can be a promising solution to remediate hydrocarbon-polluted areas, not only in developed countries but also in developing countries, in which uncontrolled disposal of oil industry wastes has polluted different resources over the past decades (Njoku *et al.*, 2009). Advantages of using phytoremediation include cost-effectiveness, aesthetic advantages, and long-term applicability (Das and Chandran, 2011).

Aquatic and wetland plants and algae can be used for the phytoremediation process under specific cases and conditions of hydrocarbon contamination (Nedunuri *et al.*, 2000; Radwan *et al.*, 2000; Siciliano and Greer, 2000; Saadoun and Al-

Ghazawi, 2005). The main mechanisms in the phytoremediation of organic contaminants are the direct uptake of contaminants and their subsequent metabolism in plant tissues, the transpiration of volatile organic hydrocarbons through the leaves, the release of exudates that stimulate microbial activity, and the enhancement of mineralization at the root-soil interface, which is attributed to the microorganisms associated with the root surface (Schnoor *et al.*, 1995; Huesemann *et al.*, 2009).

Many studies have been concentrating on the plant-microbe interaction in phytoremediation, where the presence of indigenous microorganisms can enhance the remediation efficiency of plants (Seeger *et al.*, 2011; Ahmad *et al.*, 2012; Ribeiro *et al.*, 2013). Regarding the interaction between plants and rhizobacteria, Moreira *et al.* (2011) found that the association of plants (*Rizophora mangle* L.) with the community of microorganisms in the rhizosphere enhanced the degradation of TPH (total petroleum hydrocarbons) in the sediment, with 87% efficiency, and fostered increased growth of these plants. Vieira *et al.* (2007) showed that the bacteria were able to reduce 6% diesel oil- and gasoline fuel-contaminated effluent from an initial TPH concentration of 11,000 mg L⁻¹ by 90 ± 2% over a process period of 49 days. Other studies have shown an association between plant and root biomass and the degradation of TPH (Hou *et al.*, 2001; Kaimi *et al.*, 2006). In addition, plant associated microorganisms can effectively decrease contaminant concentrations and can convert end-products into non-hazardous, inoffensive and stable substances, e.g., carbon dioxide, water and nitrogen (Alexander, 1999; Wu *et al.*, 2012). Recently, Alwan *et al.* (2013) in experimental study used the plant (*Ceratophyllum*) on pools contained water contaminated by hydrocarbon, after biodegradation by adding 5ml of coliform bacteria (*Bacillus* spp.) and concluded that the *Ceratophyllum* plant is efficient absorption of waste hydrocarbon and decrease the oil content in the water contaminated by waste hydrocarbon. Al-Baldawi *et al.* (2015) demonstrated that the *Scirpus grossus* plant has the ability to enhance diesel removal with the help of rhizobacteria and the adsorption of diesel, representing an environmentally friendly, alternative technology for the remediation of water contaminated with diesel.

Conclusion

Petroleum contamination is an environmental worry that damages aquatic ecosystems. Bioremediation is an important option for dealing with oil spills and can be used as a cleanup method for this contamination by utilizing the activities of microorganisms that occur naturally and can degrade these contaminants. This process depends on several

physical and chemical factors that need to be finely controlled to optimize the environmental conditions for the microorganisms and successfully remediate the contaminated sites. Among the used technologies for cleaning up petroleum contamination those include phytoremediation. Finally, it may be concluded that bioremediation can be considered as a key component in the cleanup technology for petroleum contaminants treatment in aquatic environments.

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