

Review Article

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Role of Microbes in Petroleum Hydrocarbon Degradation in the Aquatic Environment: A Review

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ABSTRACT

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The phenomenon of the oil spill in the marine ecosystem is one of the major issues which causes the introduction of petroleum hydrocarbon pollutants and leads to a significant threat to the environment. It can occur either naturally or from anthropogenic sources such as those caused by tanker accidents, refineries, drilling operations, or even storage facilities. This causes significant damage to both the habitat and the organisms living in the marine environment. Bioremediation by oil-degrading microorganisms is a viable option to metabolize and remove these harmful pollutants from the polluted site. Oil degrading microorganisms are ubiquitously distributed in the environment and naturally biodegrade petroleum hydrocarbon. This review highlights the mechanism of microbial degradation of petroleum hydrocarbons by enzyme catalytic activities of microorganisms to increase the rate of petroleum hydrocarbon degradation. It also highlights the factors that affect the biodegradation of petroleum hydrocarbons by the microorganisms.

Introduction

Oil pollution has been one of the major challenges to fight climate change in the world, particularly the aquatic marine ecosystem. The increase in pollution is a threat to the marine ecosystem and it also alters the balance of both the habitat and the organisms that live there. These organisms

experience altered growth and reproduction patterns, anatomical complications, and increased susceptibility to hypothermia.

The majority of the oil pollution relates to the large numbers of spills, both large and small, being recorded every year. The impact on the environment in response to oil pollution with geographical isolation and extreme

conditions, making any response logistically challenging. The Gulf War oil spill of Kuwait is the largest marine oil spill in the world (Abbriano *et al.*, 2011) that significantly threat to the marine environment. It was estimated that the Exxon Valdez oil spill of 1989 and Deepwater Horizon (DWH) oil spill of 2010 discharged 11 million gallons of Alaskan North Slope crude oil and 4.9 million barrels of South Louisiana sweet crude oil into the Gulf of Mexico respectively resulting in huge contribution in term of polluting the marine ecosystem (Bragg *et al.*, 1994). There are different techniques for the removal of pollutants and the use of microbe is a viable option as compared to conventional physico-chemical methods and became economically viable. This review will highlight the microbial degradation of petroleum hydrocarbon and also how the microbes interact with the environment regarding the mechanisms involved under aerobic conditions.

Composition of petroleum hydrocarbon

Recent advances in ultra-high-resolution mass spectrometry have allowed the identification of more than 17,000 distinct chemical components which made more complex and term as petroleomics. Crude oil can be classified into four main defined groups of chemicals: (a) the saturated hydrocarbons, (b) aromatic hydrocarbons, (c) the resins, and (d) the asphaltenes. Heavy oils contain lower saturated and aromatic hydrocarbons and a higher proportion of the more polar chemicals, the resins, and asphaltenes while light oils have higher aromatic and saturated hydrocarbons, with a smaller proportion of asphaltenes and resins. Under anoxic conditions, heavy oils result from the biodegradation of crude oil in situ in petroleum reservoirs. Due to the nature of more toxic and persistent nature, the aromatic hydrocarbons and polar fractions could be of

greater long-term environmental significance (Head *et al.*, 2006).

Microbial degradation of petroleum hydrocarbons

It has been identified that more than 79 genera of bacteria are capable to degrade petroleum hydrocarbons (Xu *et al.*, 2018). Several interesting marine bacteria that are adapted to hydrocarbon degradation have been isolated (Table 1). Hydrocarbon degrading bacteria include *Alcanivorax* spp., *Cycloclasticus* spp., *Oleiphilus* spp., *Oleispira* spp., *Thalassolituus* spp., *Planomicrobium* (formerly known as *Planococcus*) uses hydrocarbons exclusively as a carbon source. *Alcanivorax* spp., *Oleiphilus* spp., *Oleispira* spp., *Thalassolituus* spp. *Planomicrobium alkanoclasticum* MAE2 uses a variety of saturated hydrocarbons, however, *Cycloclasticus* spp. have evolved to use a range of polycyclic aromatic hydrocarbons (Head *et al.*, 2006). Wide geographical distribution of *Alcanivorax* spp., particularly in oil spills may be due to their ability to use branched-chain alkanes more effectively than other hydrocarbon-degrading bacteria, giving these species a selective advantage. Branched-chain alkanes such as pristane enter the sea from oil spills and also naturally produced by some marine plankton such as Bacillariophyceae, Cryptophyceae, Haptophyceae, and Euglenophyceae (Rontani and Bonin, 2011). Cui *et al.*, (2020) reported *Bacterium*, *Burkholderia-Paraburkholderia*, *Luteibacter*, and *Acinetobacter* mainly contributed to petroleum hydrocarbons degradation under optimal condition.

Factors influencing petroleum hydrocarbon degradation

Several limiting factors have been reported to affect the biodegradation of petroleum hydrocarbons. The interaction among

community members and the environment such as competition for limiting nutrients, predation by protozoa, lysis by phage, and cooperative interactions could increase degradation as shown in fig.1 (Head *et al.*, 2006).

Temperature

Temperature affects both the physical state of hydrocarbons and microbes residing on them (Leahy and Colwell, 1990; Chandra *et al.*, 2013). It also affects microbial growth rate, gas solubilities, soil matrix, metabolism in microbes, physical and chemical state of contaminants (Megharaj *et al.*, 2011). It was reported that an increase in temperature increases solubility of hydrophobic pollutants, decrease viscosity, enhances diffusion, and transfer long-chain n-alkanes from solid phase to water phase (Aislabie *et al.*, 2006). The viscosity of oil increases, volatilization of toxic short-chain alkanes is reduced and their water solubility is decreased which delay the onset of biodegradation at low temperature (Leahy and Colwell, 1990). Cui *et al.*, (2020) showed the degradation rates of petroleum hydrocarbons were gradually increased with increasing temperature to optimal condition.

Salinity and pressure

Salinity also hampers microbial growth and their products (Leahy and Colwell, 1990). Atlas (1981), has reported that salinity and pressure are specific features of typical ecosystems such as saline lakes or deep seas, which represent a particular environment that may be polluted by petroleum hydrocarbons. Minai-Tehrani *et al.*, (2006), have noted 41% crude oil degradation for 4 months incubation in soil samples without NaCl addition, while 12% crude oil degradation for 4 months incubation was obtained in same soil samples when 50 g/L NaCl was added. Experiments for biodegradation of hexadecane,

tetradecane, and mixed hydrocarbon substrate using a mixed culture of deep-sea sediment bacteria were performed at high pressure of 495 atm or 500 atm and atmospheric temperature of 1 atm (Schwarz *et al.*, 1975). They have concluded that hydrocarbon pollutants when reaching the deep ocean environment, some petroleum components may pollute deep benthic zones of the oceans. Varjani and Upasani (2017) suggest that these pollutants in benthic zones of oceans are biodegraded very slowly due to the recalcitrant fraction of petroleum oil that could persist for a long time may be years or decades.

Substrate and properties

Petroleum hydrocarbon mineralization is affected by its concentration (Leahy and Colwell, 1990). With increased petroleum oil concentration lags phase decreases, whereas maximum degrading rates and cumulative extents of mineralization increases (Towell *et al.*, 2011). Petroleum hydrocarbon degradability also depends on its composition (Atlas, 1981). Biodegradability of hydrocarbons can be ranked in decreasing order as: linear alkanes > branched alkanes > low-molecular-weight alkyl aromatics > monoaromatics > cyclic alkanes > polyaromatics > asphaltenes (Varjani, 2017). Immensely high complete oil hydrocarbon fixations have affirmed deadly to microbial action consequently restricting biodegradation potential (Admon *et al.*, 2001). Tremendously low total petroleum hydrocarbon concentrations can limit biodegradation as carbon supply may be too low that supports the growth of microbes (Leahy and Colwell, 1990). Typically, the biodegradation rate increases with decreasing molecular weight and chemical structure complexity of hydrocarbon (Varjani, 2017). The same compound(s) in different petroleum crude is degraded to different extents by the same or

other organisms/consortium, the reason could be bioavailability of that compound(s) (Varjani, 2017). Physicochemical properties of crude oil and polluted sites are essential for a successful bioremediation process (Varjani *et al.*, 2014b). These factors have a direct influence on the type, number, and metabolic activities of the microflora of any ecosystem (Admon *et al.*, 2001; Ghazali *et al.*, 2004). Hydrocarbon degraders prefer the utilization of less complex compounds (Atlas, 1998). Biodegradability is inherently influenced by the composition of pollutants (Ruberto *et al.*, 2003). Crude oil having major constituent as saturates and/or aromatics is biodegradable, however, for heavy asphaltic-naphthenic crude oils, approximately 11% may be biodegradable within a reasonable period under favorable growth conditions for microorganisms (Ghazali *et al.*, 2004).

Availability of nutrients

The availability of nutrients plays an important role in microbial activities (Varjani and Upasani, 2017). The type and concentration of carbon and nitrogen source used in culture medium play a vital role for microbial growth (Atlas, 1981; Jagadevan and Mukherji, 2004; Zhao *et al.*, 2011). Microorganisms require nitrogen and phosphorus for incorporation into biomass, the availability of these nutrients within the same area as hydrocarbons is critical (Atlas, 1981). Like biological oxygen demand, nitrogen demand is important for the biodegradation of hydrocarbons (Atlas, 1981). Varjani (2017), has reviewed that petroleum hydrocarbons do not contain a significant amount of some nutrients viz. such as nitrogen and phosphorous required for microbial growth. However, urea, phosphate, N-P-K fertilizers, ammonium, and phosphate salts can be used to adjust carbon/nitrogen/phosphorous/potassium (C-N-P-K) ratios. Generally, C:N:P to promote

microbial growth is 100:10:1 (Varjani and Upasani, 2017). Atlas (1995) has reported that the addition of nitrogenous fertilizers shows increased rates of petroleum hydrocarbon biodegradation. In contrast, Varjani *et al.*, (2014a) described that no significant effect in petroleum hydrocarbon biodegradation was observed on nitrogen source addition.

Toda and Itoh (2012) reported that nitrate as the best source of nitrogen for growth and biosurfactant production by microorganisms. Walworth *et al.*, (2005), reported that treating petroleum polluted site with nitrogen increases cell growth rate as well as hydrocarbon degradation rate by decreasing the lag phase of microbial growth and maintaining microbial populations at high activity levels. However, it has been shown that excessive amounts of nitrogen in soil cause microbial inhibition. Maintaining nitrogen levels below 1800 mg nitrogen/kg H₂O leads to optimal biodegradation of hydrocarbon pollutants (Walworth *et al.*, 2007). Excessive nutrient concentrations especially high concentrations of NPK levels inhibit biodegradation activity of hydrocarbon pollutants (Boopathy, 2000; Souza *et al.*, 2014). In addition to this high concentration of these pollutants in polluted sites disturb C: N: P ratio which leads to oxygen limitations (Varjani and Upasani, 2017).

Biosurfactant and bioavailability of pollutants

The bioavailability of petroleum crude pollutants to microbes plays a key role in the bioremediation process (Saeki *et al.*, 2009; Souza *et al.*, 2014). Hydrocarbon pollutants transport to microbial cells through (a) interaction of microbial cells to hydrocarbon pollutants dissolved in an aqueous phase, (b) direct contact of cells with hydrocarbons, and (c) interaction of cells with hydrocarbon droplets much smaller than cells (Goswami

and Singh, 1991; Kavitha *et al.*, 2014). Pollutant bioavailability depends on its chemical properties viz. hydrophobicity and volatility; soil properties; environmental conditions and biological activity (Pilon-Smits, 2005; Yalcin *et al.*, 2011; Varjani *et al.*, 2014a). However, solubility and dissolution rates play a critical role in bioavailability (Kavitha *et al.*, 2014). Hydrophobic, less water-soluble molecules sorb more strongly to soil components than hydrophilic molecules (Jain *et al.*, 2011). Microbial products such as acids, biosurfactants/bioemulsifiers, solvents, gases, and biopolymers also enhance bioremediation and oil recovery (Brito *et al.*, 2006; Yalcin *et al.*, 2011; Varjani and Upasani, 2016; Zhao *et al.*, 2016). Biosurfactants are secondary metabolites and produced in the stationary phase of microbial growth (Banat *et al.*, 2010).

Biosurfactants are amphiphilic molecules that may stimulate dissolution or desorption rates, solubilization, or even emulsification of hydrocarbon pollutants (Dias *et al.*, 2012; Kavitha *et al.*, 2014). It is interestingly showing about the application of microbial surfactant over chemical surfactants due to (a) ability to be synthesized from renewable feed stocks (Yan *et al.*, 2012; Zhao *et al.*, 2016) (b) relative nontoxicity and higher biodegradability (Souza *et al.*, 2014; Varjani and Upasani, 2016a), (c) unique structural properties for application in environmental clean-up (Souza *et al.*, 2014; Zhao *et al.*, 2016) and (d) higher foaming capacity, high selectivity and specific activity at harsh environmental conditions viz. temperature, pH and salinity (Varjani and Upasani, 2016). Surface active agents at low concentrations bring about changes in interfacial tension (Rabiei *et al.*, 2013). But, at increased concentration, a critical concentration reaches beyond which no change in interfacial properties is observed (Desai and Banat,

1997; Souza *et al.*, 2014). Beyond this critical concentration, surfactant molecules form micelles (Desai and Banat, 1997). Micelle formation enables surfactant to reduce surface and interfacial tension which sequentially results in increased solubility and bioavailability of hydrophobic compounds (Rabiei *et al.*, 2013; Souza *et al.*, 2014). By reducing surface tension and interfacial tension biosurfactant reduces repulsive forces between two dissimilar phases and helps these two phases to mix and interact more easily (Desai and Banat, 1997; Mulligan, 2005; Souza *et al.*, 2014). Several bacterial sp. can grow exclusively on hydrocarbons and produce biosurfactants (Mulligan, 2005). Petroleum-degrading and biosurfactant producing microorganisms are widely distributed in soil, water, and sediments (Yakimov *et al.*, 2007). Many microorganisms isolated from oil reservoirs can produce biosurfactants (Waigi *et al.*, 2015) which are of interest in bioremediation of petroleum hydrocarbon polluted sites and microbial enhanced oil recovery (MEOR).

Mechanism of petroleum hydrocarbon degradation

The rapid and complete degradation of most organic pollutants occurs under aerobic conditions. The foremost intra-cellular organic pollutant attack takes the form of oxidation and activation, and also the integration of oxygen is the key enzymatic catalyst via peroxidases and oxygenates. Das and Chandran, (2011) explained the pathways of peripheral degradation transform organic pollutants stepwise in intermediates of the central intermediary metabolism, viz. the tricarboxylic acid cycle. The cell biomass biosynthesis happens from the metabolites of the central precursors, for instance, the acetyl-CoA, pyruvate, and succinate (Das and Chandran, 2011; Al-Hawash *et al.*, 2018).

The saccharides necessary for different biosynthesis and growth are synthesized via gluconeogenesis. Petroleum hydrocarbon degradation could be possible either a) via a specific enzyme system, b) microbial cell attachment to substrates and c) biosurfactant production (Rahman *et al.*, 2003). Petroleum hydrocarbons can be selectively metabolized from an individual strain of microorganism or a microbial consortium of strains relevant to the irrespective genera (Varjani and Upasani, 2016). The consortium had shown to be more possible than the individual cultures to metabolizing or degrading of petroleum hydrocarbons (Al-Hawash *et al.*, 2018). Most recently, the degradation process of diesel by HDMP₂ could be divided into three stages such as surface adsorption, cell uptake, and biodegradation. At the initial stage, the diesel components were quickly adsorbed on the surface of HDMP₂. Then, except for little into a cell, the most diesel components were continuously gathered and finally degraded into C_nH_n, CO₂, and H₂O (Yang *et al.*, 2020).

Enzymes participating in the degradation of hydrocarbons

Cytochrome P450 alkane hydroxylases isolated from *Candida* species, including *Candida apicola*, *C. maltose* and *C. tropicalis* (Scheller *et al.*, 1998) comprise a supergroup of omnipresent Heme-thiolate Monooxygenases which assume a significant job in the microbial corruption of oil, chlorinated hydrocarbons, fuel added substances, and numerous different mixes (Van Beilen and Funhoff, 2007). Depending on the chain length, enzyme systems are required to acquaint oxygen in the substrate to initiate biodegradation (Table 2). Higher eukaryotes generally contain several different P450 families that comprise a large number of individual P450 forms that may pay as a group of isoforms to the metabolic conversion of a given substrate. Cytochrome P450

enzyme systems were engaged in biodegradation of petroleum hydrocarbons. The ability of various yeast species to utilize n-alkanes and other aliphatic hydrocarbons as a sole wellspring of carbon and energy is mediated by the presence of numerous microsomal Cytochrome P450 forms. The assorted variety of alkane oxygenase systems in eukaryotes and prokaryotes that are vigorously taking an interest in the debasement of alkanes under aerobic conditions like Cytochrome P450 enzyme, soluble di-iron methane monooxygenases, integral membrane di-iron alkane hydroxylases (e.g., *alkB*) and membrane-bound copper-containing methane monooxygenases, lipase, esterase (Das and Chandran, 2011; Kadri *et al.*, 2018).

Fig-1 shows the interaction among bacterial community members and the environment. This outline demonstrates that oil biodegradation includes progressively natural parts rather than the microorganisms that legitimately assault oil (the essential oil degraders) and shows that the essential oil degraders communicate with these segments. It is indicated that oil-degrading bacteria as a red color, solid arrow as material influxes, broken arrows as direct interactions viz. lysis by phage and predation by protozoa. For simplicity, only one function is assigned to a microorganism in this representation. However, it should be noted that a microorganism can have more than one function or ability, for instance, to weather minerals to release phosphate (P), and to degrade the oil. It should also be well-known that primary oil degraders need to compete with other microorganisms for limiting nutrients such as P and those non-oil-degrading microorganisms which are shown as a brown color that can be affected by metabolites and other compounds that are released by oil-degrading bacteria and vice versa (Head *et al.*, 2006).

Table.1 Petroleum hydrocarbon-degrading microbes and their preferred degradation substrates

Petroleum hydrocarbon components	Bacterial species	Main degrading compound	Reference
aromatic hydrocarbons	<i>Neptunomonas naphthovorana</i>	Polyaromatics	Hedlund <i>et al.</i> , 1999
	<i>Cycloclasticus</i>		Kasai <i>et al.</i> , 2002
	<i>Pseudomonas aeruginosa</i>	Monoaromatics	Mukherjee <i>et al.</i> , 2010
	<i>Mycobacterium cosmeticum</i>	Monoaromatics	Zhang <i>et al.</i> , 2013
	<i>Aeribacillus pallidus</i>	Mono-/polyaromatics	Mnif <i>et al.</i> , 2014
	<i>Achromobacter xylosoxidans</i>	Mono-/polyaromatics	Ma <i>et al.</i> , 2015
	<i>Bacillus licheniformis</i> <i>Bacillus mojavensis</i>	Polyaromatics	Eskandari <i>et al.</i> , 2017
Saturated hydrocarbons	<i>Pseudomonas</i> sp.	n-alkanes (C14–C30)	Sugiura <i>et al.</i> , 1997
	<i>Alcanivorax</i> sp.	n-alkanes and branched alkanes	Hara <i>et al.</i> , 2003
	<i>Oleispira antarctica</i>	n-alkanes (C10–C18)	Yakimov <i>et al.</i> , 2003
	<i>Rhodococcus ruber</i>	n-alkanes (C13–C17)	Zhukov <i>et al.</i> , 2007
	<i>Rhodococcus</i> sp.	Cyclohexane	Lee and Cho, 2008
	<i>Dietzia</i> sp.	n- alkanes (C6-C40)	Wang <i>et al.</i> , 2011
	<i>Geobacillus thermodenitrificans</i> <i>Gordonia sihwensis</i>	n-alkanes (C15–C36) Branched and normal alkanes	Abbasian <i>et al.</i> , 2015 Brown <i>et al.</i> , 2016
Resins	<i>Pseudomonas</i> sp.	Resins	Venkateswaran <i>et al.</i> , 1995
Asphaltenes	<i>Seudomonas</i> spp., <i>Bacillus</i> sp.	Asphaltenes	Tavassoli <i>et al.</i> , 2012
	<i>Citrobacter</i> sp., <i>Enterobacter</i> sp., <i>Staphylococcus</i> sp., <i>Lysinibacillus</i> sp. <i>Bacillus</i> sp., <i>Pseudomonas</i> sp.	Asphaltenes	Jahromi <i>et al.</i> , 2014

Table.2 Enzymes involved in the degradation of hydrocarbons

Enzymes	Substrate	Microorganism	Reference
Alkane hydroxylase, lipase, and esterase	hexane, hexadecane and motor oil	<i>Alcanivorax borkumensis</i>	T. Kadri <i>et al.</i> , 2018
Eukaryotic P450	C10–C16 alkanes, fatty acids	<i>Candida maltose</i> , <i>Candida tropicalis</i> , <i>Yarrowialipolytica</i>	Iida <i>et al.</i> , 2000
<i>AlkB</i> -related alkane hydroxylases	C5–C16 alkanes, fatty acids, alkylbenzenes, cycloalkanes, etc.	<i>Acinetobacter</i> , <i>Alcanivorax</i> , <i>Burkholderia</i> , <i>Mycobacterium</i> , <i>Pseudomonas</i> , <i>Rhodococcus</i> , etc.	van Beilen <i>et al.</i> , (2003)

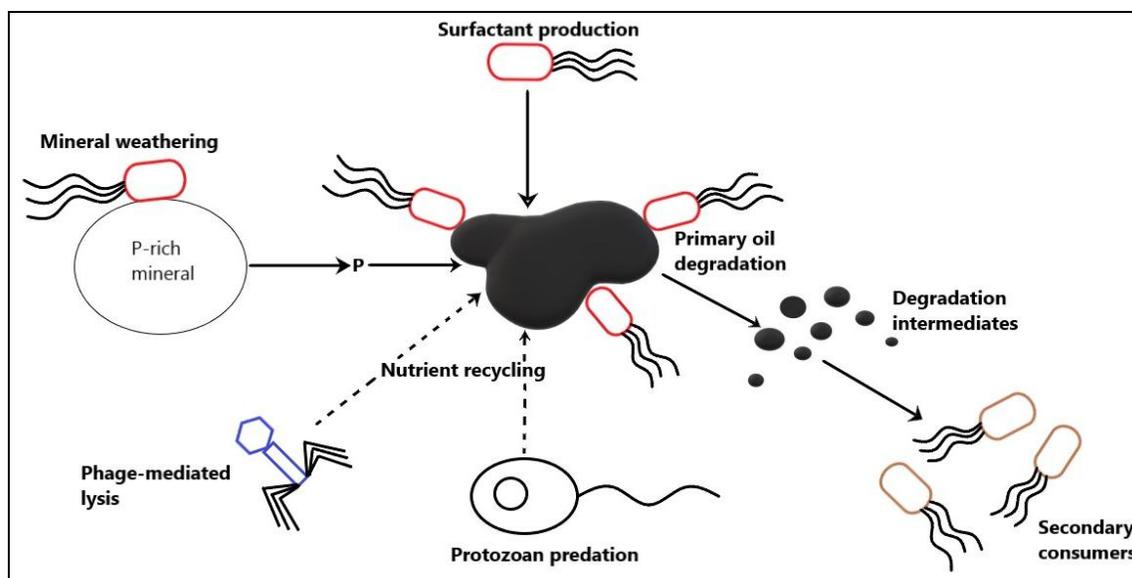


Fig.1 The interaction among bacterial community members and the environment

It is concluded as petroleum hydrocarbon pollutant in the marine environment is one of the major concern to be addressed to mitigate by using any possible ways. Biodegradation of persistent organic pollutants can be performed using oleophilic microorganisms either as an individual organism or consortium of microorganisms to control environmental pollution. Understanding factors affecting biodegradation is of great research interest. Additional studies could be carried out to compare the performance or efficiency of isolated strains.

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