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Principles of microbial degradation of petroleum hydrocarbons in the environment

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ABSTRACT

Petroleum hydrocarbons (PHs) are a big group of chemicals that have caused a major concern because of their widespread distribution into the environment, bioaccumulation potential, harmful effects and biodegradation resistance. Soil and water pollution is mainly attributed to hydrocarbons from oil refineries, petrochemical industries, human activities and other sources. The mechanisms and factors that affect biodegradation should be further understood because the choice of bioremediation technique depends on them. This review described fungal PHs degradation, emphasized the relevant physicochemical and biological factors, and discussed the enzymatic systems influencing PHs biodegradation.

Contents

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Introduction

Petroleum hydrocarbons (PHs) are considered the main energy source and materials for different industries (Varjani and Upasani, 2016a). Many threats exist in the environment when PHs are used as energy sources. PHs are major environmental pollutants generated by wide-scale production, transport, coastal oil refining, shipping activities, offshore oil production and accidental spilling (Arulazhagan et al., 2010). Human activities, such as municipal run-offs and liquid release and industrial, cause PH pollution which impacts the environment and poses a direct or indirect health hazard to forms of life (Sajna et al., 2015). In an accidental leak, on-site removal, treatment or recovery of contaminants is facilitated but contaminants in petrol stations and spills may persist because the amount of leakage is small. PH leakage due to frequent accidental and illegal disposal of oil waste at sea severely harms various ecosystems. PHs are toxic compounds classified as priority pollutants (Costa et al., 2012). Aliphatic and aromatic hydrocarbons are two major PH components that have been reported because they are recalcitrant and harmful to health. Aliphatic hydrocarbons are easily degraded by microorganisms, but large branched aliphatic chains are not easily degraded; therefore, they persist in the environment (Hasanuzzaman et al., 2007). Likewise, aromatic hydrocarbons are difficult to degrade because of their complex structures. In vitro and in vivo experiments have showed that polycyclic aromatic hydrocarbons (PAHs) are carcinogenic, cytotoxic, genotoxic and environmentally toxic. PAHs are fused aromatic ring compounds found in the atmosphere and relatively resistant to the biodegradation; as such, they accumulate to significant levels into the environment (Freeman and Cattell, 1990). A biological treatment is an alternative pollutant removal method because this technique does not elicit deleterious effects on the environment. This treatment may also be less expensive than other techniques. The success of bioremediation depends integrally on pollutant biodegradation, pollutant-degrading organism accessibility and biological activity optimization. Biodegradation by indigenous microorganisms is a major mechanism and a reliable method that operates by biologically removing foreign contaminants, such as crude oil (Ghanavati et al., 2008). Bacteria, yeast and fungi can utilize PHs (Haritash and Kaushik, 2009). Fungi such as *Aspergillus, Penicillium, Fusarium, Amorphotheca, Neosartorya, Paecilomyces, Talaromyces, Graphium Cunninghamella* are microorganisms which can degrade persistent pollutants, see Table 1. This review provides current information on PH degradation by fungi to enhance our understanding of bioremediation challenges.

Degradation of PHs by microbial activity

PHs degradation is a very hard method that influenced mainly in the amount and nature of the PHs present. PHs divided in four categories which are as follows: aliphatics, aromatics, resins (carbazoles, sulfoxides, pyridines, quinolines and amides) and asphaltenes (phenols, ketones, esters, porphyrins and fatty acids,) (Steliga, 2012). Numerous studies have reported that different environmental factors influence the biodegradation of PHs (Cooney et al., 1985). The limited availability of microorganisms in the environment is one of the most significant factors that restrict biodegradability of oil contaminants. Bioremediation of sites polluted with crude oil are oftentimes limited because of the poor biodiversity of local microbes or the scarcity of local specialized microbes with supplementary substrate properties required for the degradation of various hydrocarbons present in contaminated sites (Ron and Rosenberg, 2014) Some of the PAHs with a high molecular weight are probably not degraded at all. Degradation of Microbial is a major and a final natural mechanism which can help to clean-up PH contaminants in the environment (Juhasz and Naidu, 2000). Bacteria and filamentous fungi participate in the PH biodegradation (Rahman et al., 2003). In the past few years the biodegradation of ligninolytic fungi has been studied. Major enzymes in the lignin system including lignin peroxidases, manganese-dependent peroxidases, phenol oxidases (laccases and tyrosinases) and H2O2-producing enzymes, have been proven to degrade PAHs (Lee et al., 2015). Many species of fungi have been proven to have a high potential for PH degradation. Furthermore, numerous fungi are naturally living on soil waste and can be grown in soil and propagated during a solid matrix to remove PHs. Those criteria indicate to the environmental fungi role in bioremediation (Lee et al., 2015).

Table 1
PHs degradation by different species of fungi.

Fungi	Compound	References
Trichoderma harzianum	Naphthalene	Mollea et al. (2005)
Aspergillus fumigatus		Ye et al. (2011)
Aspergillus spp	Crude oil	Zhang et al. (2016)
Cunninghamella elegans	Phenanthrene	Romero et al. (1998)
Aspergillus niger	n-hexadecane	Volke-Sepúlveda et al. (2003)
Penicillium sp		Pointing (2001)
Cunninghamella elegans	Pyrene	Cerniglia and Yang (1984)
Aspergillus ochraceus	Benzo[a]pyrene	Passarini et al. (2011)
Trametes versicolor		Collins et al. (1996)
Penicillium sp. RMA1 and RMA2	Crude oil	Al-Hawash et al. (2018a)
Aspergillus sp. RFC-1	Different PHs	Al-Hawash et al. (2018b)

Factors influencing the degradation of PHs

Many studies and successful applications have been done in the treatment of contaminated soil and water. A comprehensive study on pollution caused by PHs and bioremediation methods has also been performed. The activity of microbial can be affected by the following factors: temperature, oxygen, pH and nutrients, see Fig. 1. For successful biodegradation, microorganisms should develop a catabolic activity by the following processes: new metabolic capabilities development by changes of genetic, induction of specific enzymes and eclectic enrichment of microorganisms that are capable to convert the pollutants. When conditions are favorable to the microorganisms, biodegradation of PHs will reach a maximum level. The chemical composition of the PHs is the fundamental and influential factor in biodegradation. Fedorak and Westlake (1981) reported that the aromatic hydrocarbons were attacked more quickly during the crude oil degradation by marine microbial populations. Rambeloarisoa et al. (1984) used a continuous culture fermenter and a mixed culture of marine bacteria, and showed that the degradation of all fragments of crude oil was at

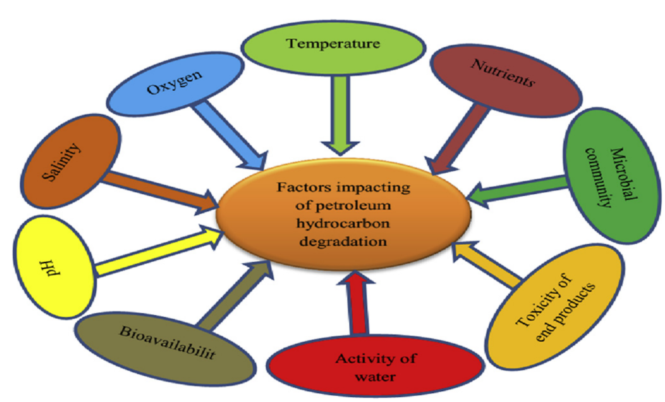

Fig. 1. Factors affecting in biodegradation of petroleum hydrocarbons.

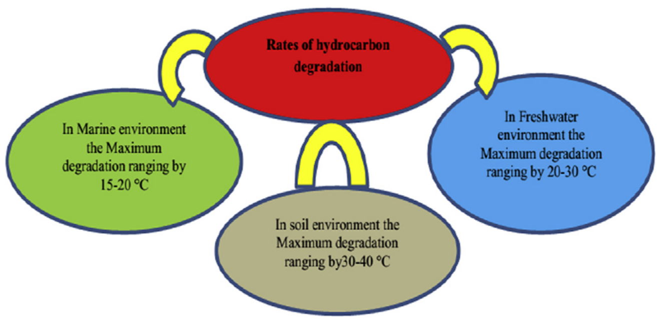

Fig. 2. Hydrocarbon degradation rates in soil, fresh water, and marine environments.

the same rate. All of the saturates, aromatics, resins and asphaltenes varied greatly in degradation (Jobson et al., 1972). PHs that leaked in water tend to spread and form the spot due to the wind and wave action; water in oil or oil in water ("foam") may form emulsions (Colwell et al., 1977). An important process in the absorption of PHs by microorganisms is the formation of an emulsion by their own production or by the help of biosurfactants. Singh and Ward (2004) demonstrated the main differences between the biodegradation of the oil in the soil and in the aquatic environment after oil spill. The movement and the allocation of the oil and the existence of particles affect its physical and chemical nature which consequently affects the microbial degradation.

Temperature

Temperature is among the factors that influence PH biodegradation by affecting the physical and the chemical compositions of PHs (Atlas, 1981). At low temperatures, the degradation rate is generally observed to decrease, which is thought to be a result of reduced enzymatic activity rates (Bisht et al., 2015). The rate of hydrocarbon metabolism reached the maximum level in high temperatures ranging from 30 °C to 40 °C (Bossert and Bartha, 1984). Despite biodegradation of hydrocarbons can take place on a wide domain of temperatures, degradation rate decrease through declining temperature. The highest rates of degradation that occurred at the temperature range of 30–40 °C, 20–30 °C and 15–20 °C in soil, marine and freshwater environments, respectively, (Atlas, 1985) see Fig. 2. Colwell et al. (1978) proved that the Metula crude oil degradation through mixed cultures of marine bacteria is possible at 30 °C. Leahy and Colwell (1990) reported that the biodegradation of petroleum in soil occurs at −1.1 °C. PAH biodegradation in estuarine sediment is limited at low winter temperatures (Shiaris, 1989).

Oxygen

The concentration of oxygen has been determined as the rate-limiting variable for PHs degradation in the environment (Von Wedel et al., 1988). The oxygen availability in the soil depends on microbial oxygen consumption rates and soil type, whether soil is water-logged, and the useable substrates presence which can drive to oxygen depletion(Haritash and Kaushik, 2009) . Some studies have indicated that anaerobic degradation of PHs by microorganisms can happen at negligible rates (Haritash and Kaushik, 2009). As confirmed in recent studies, microbial consortia of sludge and soil have been able of metabolizing alkyl-substituted aromatics and unsubstituted to benzene, 1,3-dimethylbenzene, and acenaphthene, naphthalene, toluene and xylene, in the molecular oxygen absence (Grbić-Galić and Vogel, 1987). McNally et al. (1998) reported that the aerobic biodegradation of PHs was higher compared with the anaerobic biodegradation. Biodegradation of PHs in anaerobic conditions was not as fast in aerobic conditions (Grishchenkov et al., 2000). Substrate oxidation by oxygenases in the catabolism of all aliphatic, cyclic and aromatic compounds by microbial is considered a key step in the biodegradation process (Meng et al., 2017).

Nutrients

Nutrients are important components for a successful the contaminants biodegradation, including nitrogen, iron and phosphorus in some cases (Atlas, 1985). Some of those nutrients can become a limiting factor thus impacting in the processes of biodegradation. Carbon comes from an organic source (PHs); hydrogen and oxygen are supplied from the water (Kalantary et al., 2014). In marine and freshwater environments, oil spills cause a dramatic increase of carbon levels and a decrease of nitrogen and phosphorus levels which can affect the biodegradation process. In marine environments, nitrogen and phosphorus levels are low, and the wetlands are unable to provide the nutrients because of strong demands of nutrients by the plants. Thus, nutrients addition was necessary to promote the biodegradation of contaminants (Hesnawi and Adbeib, 2013). On the other hand, the concentration of excess nutrients can also inhibit the activity of biodegradation (Atlas, 1985). Zafra et al. (2015) observed that the pollutants concentration had a selective pressure on petroleum-degrading organisms, the high PAH levels were limiting the growth of microorganisms that developed a response against PAHs, concerning the structure of cell membrane, alterations of sporulation and mycelia pigmentation. Balaji et al. (2014) also examined various sources of carbon for lipase production by *Penicillium chrysogenum*, *Lasiodiplodia theobromae* and *Mucor racemosus*, and found that sucrose and cellulose induced the highest activity in those species. Likewise, sources of nitrogen should be taken into consideration. Yeast extract was the better catalyst of high level production of lipase in the above-mentioned strains. Furthermore, Mineki et al. (2015) studied PAHs degradation by *Trichoderma/Hypocrea* using pyrene as a carbon source, after addition of 0.02% yeast extract, 0.1% lactose or 0.1% sucrose, the strain growth and pyrene-degrading efficiency were enhanced compared with the control after 7 and 14 days of incubation.

Salinity

A positive relationship exists between salinity and mineralization rates of PAHs in estuarine sediments (Kerr and Capone, 1988). Ward and Brock (1978) reported the evaporation of salt ponds, which indicates that the hydrocarbon metabolism rates were greatly reduced with the increase of salinity in the range of 3.3%–28.4% due to a general decline in microbial metabolic rates. Qin et al. (2012) suggested that the salinity had a major influence on bioremediation and biodegradation process, and it also affects microbial growth and diversity. Salinity has an adverse influence on the activity of some key enzymes complicated in the process of hydrocarbon degradation (Ebadi et al., 2017).

pH

The pH can be highly variable and must be taken into consideration when improving biological treatment methods. The environmental pH affects processes such as cell membrane transport and catalytic reaction balance well as enzyme activities (Bonomo et al., 2001). Most of the heterotrophic bacteria prefer to grow in a neutral to alkaline pH in contrast to the pH of the most aquatic ecosystems, and soil acidity can highly vary, ranging 2.5–11 pH in alkaline deserts (Bossert and Bartha, 1984). In general, heterotrophic fungi and bacteria prefer a nearly neutral pH, although fungi are tolerant to acidic conditions. Hambrick et al. (1980) found that microbial mineralization of naphthalene and octadecane was present at a pH of 6.5. Rates of octadecane mineralization increase remarkably when pH increases from 6.5 to 8.0, whereas the mineralization rate of naphthalene remains unchanged. .Thavasi et al. (2007) found that the maximum biodegradation of crude oil by *Pseudomonas aeruginosa* in water was at pH 8.0. Dibble and Bartha (1979) also found the maximum biodegradation rate at pH 7.8 in oil sludge samples. Pawar (2015) observed that the soil pH 7.5 was most convenient for the degradation of all the PHs. The degradation of Phenanthrene in liquid media was favorable at a range of pH values (pH 6.5–7.0) by *Burkholderia cocovenenas*, isolated from a petroleum-polluted soil.

Activity of water

The biodegradation of hydrocarbons in terrestrial ecosystems may be restricted because of the water available for metabolism and growth of microbial. Dibble and Bartha (1979) showed that biodegradation was optimal with 30–90% water saturation in oil sludge. Atlas (1981) had suggested that the tar balls that were deposited on beaches may represent another case of limitation of Microbial degradation to hydrocarbon. Availability of water directly impacts the movement and microorganism's growth.

Microbial community

Bacteria, yeast, fungi and some algae can degrade PHs. One of the main factors impacting PHs degradation is the availability of microorganisms that can catabolise pollutants. Furthermore, bacteria and also fungi contribute to the degradation of hydrocarbons in the soil Atlas (1985). Microorganisms use PHs as sources of food that can be easily found in enormous amounts near places exposed to oil contamination, such as crude oil seeps, shipping lanes, ports, oil fields, gas stations and other similar facilities.

Bioavailability

Bioavailability refers to the portion of a chemical in soil, which can be taken up or transformed by living organisms. Bioavailability has also been defined as the influence of the physical, chemical and microbiological factors to the extent and rate of biodegradation. The pH, the microbial community and the extent of deterioration of the hydrocarbon can be significantly affected by the restrictions in the bioavailability of hydrocarbons. The bioavailable part of the hydrocarbons is the area accessible to microorganisms. PHs have low bioavailability and are classified as hydrophobic organic pollutants. Those chemicals have little water solubility, which makes them resistant to photolytic breakdown and chemical biological (Semple et al., 2003).

Toxicity of end products

The biological treatment principle is to put an end to toxins and pollutants from the restricted environment by using microorganisms. Recently, using a bioreactor for treat contaminated PAH gas-work soil evaluated both PAHs accumulation and removal of oxy-PAHs, such as coumarins, quinones and PAH-ketones (Lundstedt et al., 2003). These compounds are formed through the metabolism of microbial to PAHs and can also be configured by photo-transformation of PAHs and chemical oxidation (Kochany and Maguire, 1994).

Degradation mechanism of PHs

The rapid and complete degradation of most organic contaminants occurs under aerobic conditions. The first 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. Pathways of peripheral degradation transform organic contaminants step by step in intermediates of the central intermediary metabolism, for instance, the tricarboxylic acid cycle. The cell biomass biosynthesis happens from the metabolites of the central precursors, like the acetyl-CoA, pyruvate and succinate. The saccharides necessary for different biosynthesis and growth are synthesized via gluconeogenesis. PH degradation could be possible via a specific enzyme system. Other mechanisms are also implicated, such as microbial cell attachment to substrates and biosurfactant production (Rahman et al., 2003). PHs can be selectively metabolized from an individual strain of microorganisms or a microbial consortium of strains pertinence to the same or dissimilar genera (Varjani and Upasani, 2016b). The consortium had showed to be more possibility than the individual cultures to metabolizing or degrading of PHs (Varjani and Upasani, 2016a)

Enzyme's role in PHs degradation

Cytochrome P450 hydroxylases is participates in the microbial degradation of chlorinated oil, PHs and other compounds (Van Beilen and Funhoff, 2007). The cytochrome P450 enzymes had isolated from Candida species, including *Candida apicola*, *C. maltose* and *C. tropicalis* (Scheller et al., 1998). Alkane oxygenases, like cytochrome P450 enzymes, integral membrane di-iron alkane hydroxylases (e.g., alkB), membrane-bound copper-containing methane monooxygenases and soluble di-iron methane monooxygenases, are diverse in prokaryotes and eukaryotes and are actively implicated in the alkanes degradation under aerobic conditions (van Beilen and Funhoff, 2005). Fungi are efficient options for PH degradation, fungi have several advantages over bacteria because of their ability to cultivate on a large group of substrates. They also produce extracellular enzymes, which can penetrate contaminated soil and remove pollutants (Messias et al., 2009). The biodegradation efficiency and level of pollutants by fungal enzymes rely on growth factors, like nutrient accessibility, oxygen and optimal enzyme conditions, including pH, temperature, chemical structure, chemical partitioning in growth media and cellular transport properties (Singh and Ward, 2004). Fungi have evolved because of the irregular structure of lignin and have improved their efficiency to degrade and mineralize different organic pollutants. The extracellular peroxidases of those fungi are answerable to the initial oxidation of PHs (Zhang et al., 2015). Fungal lignin peroxidases directly oxidize several PHs, whereas manganese peroxidases of fungal indirectly co-oxidize them via enzyme-mediated lignin peroxidation (Li et al., 2014). Novotný et al. (2004) examined the LiP, MnP and laccase enzymatic activities and the degradation of pyrene and anthracene by different ligninolytic fungal species cultivated in liquid and soil. The degradation of pyrene and anthracene by *Trametes versicolor, Pleurotus ostreatus and Phanerochaete chrysosporium,* depends on levels of MnP and laccase secreted in the soil (Novotný et al., 2004). Therefore, the fungal PAH degradation is not as effective or as fast as the bacterial PAH degradation, but they are non-specific and able to hydroxylate various xenobiotics. Lactase, LiP and MnP, other fungal enzymes, including epoxide hydrolases, cytochrome P450 monooxygenase, dioxygenases, proteases and lipases, have been widely investigated because of their ability to degrade PAHs (Balaji et al., 2014). The system of extracellular enzymes from six *Aspergillus* species, isolated from crude oil-polluted soil, efficiently degrades crude oil and shows potential for crude oil recovery (Zhang et al., 2016). Jové et al. (2016) evaluated the anthracene degradation efficiency of three non-ligninolytic and ligninolytic fungi, observed that the anthracene degradation efficiency of *Phanerochaete chrysosporium* is higher than those of *Pleurotus ostreatus* and *Irpex lacteus*. Balaji et al. (2014) analyzed the ability of various fungal species to produce extracellular enzymes, like laccase, lipase, protease and peroxidase.

Conclusion and recommendations

The removal of PHs in sub-surface environments is a global problem. Biotic and abiotic factors, including nutrition, physical conditions, diversity of microbial communities involved and bioavailability of substrates, play an important role in the biodegradation process of PH-contaminated soils and water systems. Microbial degradation helps eliminate oil leakage from the environment after large quantities of oil are removed through various physical and chemical methods. These methods show potential because microorganisms possess enzyme systems to degrade and use various PHs as a sole carbon and energy source. Therefore, degradation of microbial can be considered a key process of pH remediation. The increases in our understanding of the microbial petroleum-degrading communities and the mechanisms by which petroleum biodegradation occur will prove helpful for predicting the environmental fate of these compounds and for developing practical PH bioremediation strategies in the future. It is crucial to continue in developing a technology which is cheap, easy to handle and feasible and can clean up the oil spills and other contaminated environment. Further study could be conducted to compare the performance or efficiency of strains isolated. The application of this technology in degrading hydrocarbon polluted water resources can also be looked at.

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