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Principles of Fungi Capacity in Petroleum Hydrocarbons Degradation

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ABSTRACT:
Petroleum hydrocarbons (PHs) are a large group of chemicals. They represent a major concern because of their widespread spread in the environment, their potential to bioaccumulate, their harmful effects and their resistance to biodegradation. In the current picture of the world, major environmental pollution in soil and water is attributed to the pollution of hydrocarbons from oil refineries, petrochemical industries, human activities and so on. A better understanding of the mechanisms and factors that affect biodegradation is of great environmental importance because the choice of bioremediation technic depends on it. Whereas many reviews have focused on metabolic and ecological aspects of PHs degradation, this review exhibited the fungi PHs-degradation with special emphasis on both physicochemical and biological factors influencing the biodegradation.
Keyword: *petroleum hydrocarbons; biotic and abiotic factors; biodegradation and bioremediation Fungi*

INTRODUCTION

Petroleum hydrocarbons (PAHs) are considered as a main source of energy. There are a lot of threats to the environment with using this energy. PHs are major pollutants of environments, wide-scale production, transport, coastal oil refineries, shipping activities, offshore oil production and accidental spills [1]. During an accidental leak, the procedures will be taken for the removal, treatment, or recover contaminants on the spot, while in petrol stations and spills may continuous long-term due to that the leakage a little. PHs leakage of accidental and illegal disposal frequent of oily waste at sea all of these lead to serious harm to the ecosystems [2]. Two major parts of PHs that are reported for their recalcitrant and health hazardous nature are aliphatic and aromatic hydrocarbons. An aliphatic part is easily degraded by microorganism but with large branched chains is difficult-degradable and therefore persist in the environment [3]. The aromatic fraction with their complex structures the degradation has been difficult. Polycyclic aromatic hydrocarbons (PAHs) are pollutants of wide range, many studies conducted in vitro and vivo they have been found to be carcinogenic, cytotoxic, genotoxic and environmental toxicity [4]. PAHs are fused ring aromatic compounds, everywhere pollutants in the atmosphere and relatively resistant to biodegradation, therefore accumulate to substantial levels in the environment [5, 6]. All of the crude oil and heavy oil are containing of PAHs, and also could easily form by the lack of complete combustion of organic compounds that including the fossil and bush fires and burning of vegetation in a forest [7]. Biological treatment is an alternative for the treatment of Pollutants because there is no impact of the deterioration of the environment. It may also be less expensive compared to other techniques. The bioremediation success depends on an integral of pollutant biodegradation, and accessibility of the pollutants degrading organisms and optimizing the biological activity [8]. Biodegradation by indigenous microorganisms are a central mechanism and the most reliable that operate to exclusion of foreign pollutants biologically, including crude oil are eliminated from the environment [9]. A wide range of bacteria , yeast and fungi are capable of exploiting hydrocarbons [10]. Fungi one of microorganism that have capable of degradation of persistent pollutant, such as fungi belonging to the genera *Aspergillus*, *Penicillium*, *Fusarium*, *Amorphotheca*, *Neosartorya*, *Paecilomyces*, *Talaromyces*, *Graphium* and *Cunninghamella*. This review provides an updated information on fungi degradation of PHs contaminants through the better understanding in bioremediation challenges.

2- Degradation of PHs by the microbial activity

Biodegradation of PHs is a very complex process that depends mainly on the nature and on the amount of the hydrocarbons present. PHs contains are four fragments: the aliphatic , the aromatics, the resins (carbazoles, sulfoxides, pyridines, quinolines, and amides) and the asphaltenes (phenols, fatty acids, ketones, esters, and porphyrins), [11]. Numerous studies reported that the different environmental factors are influencing in the biodegradation of PHs [12]. The limited availability of microorganisms in the environment is one of the most important factors that restrict biodegradability of oil pollutants. Differ the ability of microbial to PHs degradation, which is can be as follows: linear alkanes > branched alkanes > small aromatics > cycloalkanes [13, 14]. some of PAHs with a high molecular weight probably not be degraded at all [15] . Microbial degradation is a major and final natural mechanism, that can using to cleanup PH-contaminants from the environment [15]. Bacteria are most active , and also filamentous fungi participate in the biodegradation of PHs [16, 17]. The biodegradation is dependent on the microorganisms, the contaminant type and concentration. In the past few years the biodegradation of ligninolytic fungi has been studied intensively [18]. Extracellular enzymes are produce from lignolytic fungi due to the irregular structure of lignin, that making them suitable for degradation of different compounds. Three major enzymes in the lignin system including lignin peroxidase, manganese dependent peroxidase, phenol oxidases (lacases, tyrosinase), and H2O2-producing enzymes, numerous studies have proven that lignolytic enzymes are be able to degrade PAHs [19]. Many species of fungi have been proven a highly potential for PHs degradation.

3- Factors impacting to the degradation of PHs

Many studies and successful applications have been done in the treatment of contaminated soil and water. It was done a comprehensive study of pollution caused by PHs and bioremediation methods. When conditions are favorable to the microorganisms will reach a maximum level in the biodegradation of the PHs. The Chemical Composition of the PHs is the fundamental factor and influential in biodegradation. Rates of biodegradation have shown be greater for the saturates, followed light aromatics, with a high molecular weight aromatic and polar compounds show very low rates of degradation[20-22]. On the contrary, based on a study by Cooney et al (1983), recorded a high level of the degradation of naphthalene than of hexadecane in water-sediment mixtures from a freshwater[20]. Fedorak and Westlake reported that the aromatic hydrocarbons a more fast attack during the degradation of crude oil by marine microbial populations [23]. Bertrand et al (1984), had used continuous culture fermenter and a mixed culture of marine bacteria. They observed degradation of all fragments of crude oil at the same rates [24]. All of the saturated, aromatic, resin, asphaltene were varied greatly in degradation [22]. PH leaked in the water tend to spread and form the spot, due to the wind and wave action, oil-in water or water in- oil ("foam") may form of emulsions [25]. PH dispersion in the water column in the form of oil- in-water emulsions increase the surface area of oil, and therefore it available for the attack of microbial. Nevertheless, large plates of mousse establish unfavorably low surface-to-volume ratios, inhibiting biodegradation [26]. Troubles, that is big aggregates of weathered and undegraded oil, due to their limited surface area, therefore, it restricts access by microorganisms [27]. Emulsions formation through the production of microbial and the biosurfactants a very important process in the absorption of hydrocarbons by bacteria and fungi [28]. Singh et al demonstrated the main differences between the biodegradation of the oil in the soil and the aquatic environment after oil spill [29]. The movement and distribution of oil and the presence of particles are affecting the physical and chemical nature of the oil and thus, affect to microbial degradation [29].

3-1 Temperature

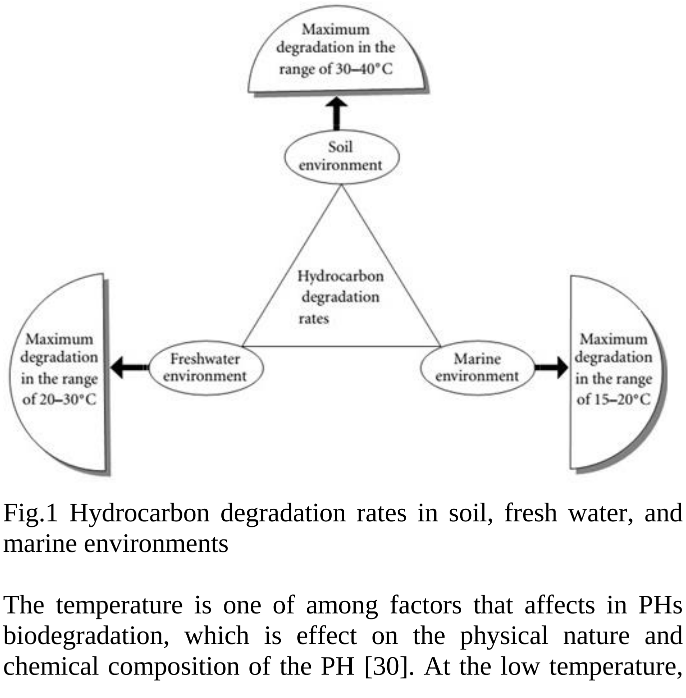

Fig.1 Hydrocarbon degradation rates in soil, fresh water, and marine environments

The temperature is one of among factors that affects in PHs biodegradation, which is effect on the physical nature and chemical composition of the PH [30]. At the low temperature, degradation rate is generally observed to decrease, which is thought to be a result of reduced enzymatic activity rates [31]. Observed Increasing rates of hydrocarbon metabolism to a maximum with high temperatures that in the range of 30-40 °C [32]. Although the biodegradation of the hydrocarbons that can happen on the wide range of temperatures, the rate of biodegradation decreases with decreasing of temperature. The highest rates of biodegradation are occur at 30-40◦C scale in soil environments, 20-30 ◦C in some fresh water environments and 15-20 ◦C in the marine environments, Fig .1 [33, 34]. Colwell et al., proved that the degradation of Metula crude oil through mixed cultures of marine bacteria in the 30°C [27]. Other study by Huddleston and Cresswell observed biodegradation of petroleum in soil at -1.1°C [17]. PAHs biodegradation in estuarine sediment was limited at low winter temperatures [35].

3-2 Oxygen

The oxygen concentration has been identified as the variable of rate-limiting in the petroleum biodegradation in soil [36]. The oxygen availability in the soil depends on rates of microbial oxygen consumption, and the type of soil, whether water-saturated soil, the presence of substrates for use which can lead to oxygen depletion [32]. Some studies have been indicated anaerobic degradation of PHs by microorganisms that happen just at negligible rates [37]. As confirmed in recent studies that microbial consortia from oil sludge have been capable of metabolism unsubstituted and alkyl-substituted aromatics, including benzene, 1,3-dimethylbenzene, acenaphthene, and naphthalene, toluene, xylene, with the absence of molecular oxygen [38]. McNally et al. reported that the aerobic biodegradation of PHs that be owned by a level higher than that anaerobic biodegradation [39]. Molecular oxygen acts as the electron acceptor if oxygen is not existing aerobic biodegradation will not occur [40]. All of the nitrate, sulfate, and iron can serve also as electron acceptors. However, in general, biodegradation of hydrocarbons does not take place in anaerobic conditions as faster as it does in aerobic conditions [41]. Oxidation of the substrate by oxygenases in the catabolism of all the aliphatic, cyclic and aromatic compound by bacteria and fungi considered a key step in biodegradation process [28]. Thus, aerobic conditions are needful for this route of microbial oxidation of the environment.

3-3 Nutrients

Nutrients are very significant ingredients for successful hydrocarbon biodegradation pollutants including nitrogen, phosphorus, and iron in some cases [42]. Some of these nutrients may become a limiting factor that impacting the biodegradation process. Carbon is provided from a source of organic (PHs); hydrogen and oxygen are supplied from the water [34]. Nitrogen, phosphorus, and sulfur are provided from inorganic sources, while the other elements such as potassium, calcium, manganese, iron, cobalt, and zinc are supplied from inorganic salts [40]. All of the components that make up the microbial biomass, it must be available in suitable concentrations for biodegradable. Oil spills in the marine and freshwater environments, provision of carbon dramatically, but nitrogen and phosphorus are limiting factor for the biodegradation. In marine environments, levels of nitrogen and phosphorus are low, and the wetlands inability to provide the nutrients due to strong demands of nutrients by the plants. So, additions of nutrients were necessary to promote the biodegradation of oil pollution [43]. On the other hand, the concentration of excess nutrients can also inhibit the activity of biodegradation [34].

3-4 Salinity

There is a positive relationship between salinity and mineralization rates of naphthalene and phenanthrene in sediments of estuarine, and also observed a correlation between the rate of naphthalene mineralization and salinity [44]. Ward and Brock (1978) reported that the evaporation of salt ponds, shown the hydrocarbon metabolism rates was a great decreased with the increase of salinity in the range of 3.3 up to 28.4%, that attributed due to a general decline in microbial metabolic rates [45].

3-5 PH

The PH can be highly variable and must be taken into consideration when improving bioremediation. The environment pH affects processes such as cell membrane transport and balance of catalytic reactions [12]. Most of the heterotrophic bacteria prefer to grow in a neutral to alkaline pH, the contrast to most aquatic ecosystems, and it can be soil acidity highly variable, ranging from 2.5-11 in alkaline deserts [32]. In general, heterotrophic bacteria and fungi prefer a pH near neutral, fungi being more tolerant of acidic conditions. Observed the optimum pH of 7.8, in the range of 5.0 to 7.8, for mineralization oily sludge into the soil [46]. Hambrick et al. (1980) found microbial mineralization of rates of octadecane and naphthalene with a pH of 6.5, rates of mineralization octadecane increased greatly when the pH was raised from 6.5 to 8.0, while the rates of naphthalene mineralization are not [46].

3-6 Activity of water

The biodegradation of hydrocarbons in terrestrial ecosystems may be restricted because of the available water for microbial growth and metabolism. Dibble and Bartha (1980) reported that the biodegradation was optimum with 30-90% water saturation in oil sludge [47]. Atlas., (1981) had suggested that the tar balls deposited on beaches may represent another case in that available water limits biodegradation of hydrocarbon [30]. Availability of Water directly affects the movement and microorganisms growth. Oil biodegradation occurs at the interface of oil and water from the oil for free and in the dissolved phase. All requirements of the cell are transported from the aqueous environment into in cell [48]. The moisture content in the soil affects various physical processes, availability of pollutants, food, and transport of gasses[40].

3-7 Microbial Community

Bacteria, fungi and yeast, as well as some algae, have an ability to degrade PHs. Individual microbial species ability metabolize only a limited a group of hydrocarbon substrates, therefore, a consortium of organisms is required for Metabolism of the complex mixture of hydrocarbons. Among the main factors affecting degradation of PHs is an availability of microorganisms that have the ability catabolic to degrade pollutants, all of the bacteria and fungi contribute to the degradation of hydrocarbon in the soil [34]. Microorganisms that use PHs as a source of food that can be easily found in enormous amounts near places exposed to oil pollution, such as crude oil seeps, shipping lanes, ports and oil fields, gas stations, and similar facilities. Abiotic factors, being the ones mentioned above and others especially with the local environmental conditions of each site have a selective effect on degrading community.

3-8 Bioavailability

The term ‘bioavailability’ refers to the portion of a chemical in soil, which can be transformed or taken up by living organisms. Bioavailability has also been defined as the impact of the physical, chemical and microbiological factors on the rate and the extent of biodegradation. The pH and microbial community degrees rate and the extent of the deterioration of the hydrocarbon can be significantly affected by the restrictions in the bioavailability of hydrocarbons. Bioavailable part of the hydrocarbons is the part accessible to microorganisms. PHs have a low bioavailability and are classified as the hydrophobic organic pollutants. These chemicals are a little water solubility, which is resistant to the biological, chemical, and photolytic breakdown [49]

3-9 Toxicity of products end

The biological treatment principle is tantamount to put an end to toxins and pollutants from the restricted environment using microorganisms. Most commercial biological treatment trials tend to monitor the successful treatment by the degree of removal of the parent polluted and do not quite work out into consideration the possibility of the biological production of more toxic metabolites. However, it is important to ensure that contaminated material is suitable for toxicity at the end of treatment [50]. Recently, using a bioreactor to treat PAH-contaminated gasworks soil monitored both the removal of PAHs and the accumulation of oxy-PAHs, such as coumarins, quinones, and PAH-ketones [51]. These compounds are formed through the microbial metabolism of PAHs and can also be formed by chemical oxidation and phototransformation of PAHs [52]. These transformation products can be evenly toxic, if not more toxic to the health of human when compared with the parent PAH. With many of the oxy-PAHs formed during treatment of PAH soil-contaminated more stable than the parent compounds[51].

4. Degradation mechanism of Petroleum hydrocarbon

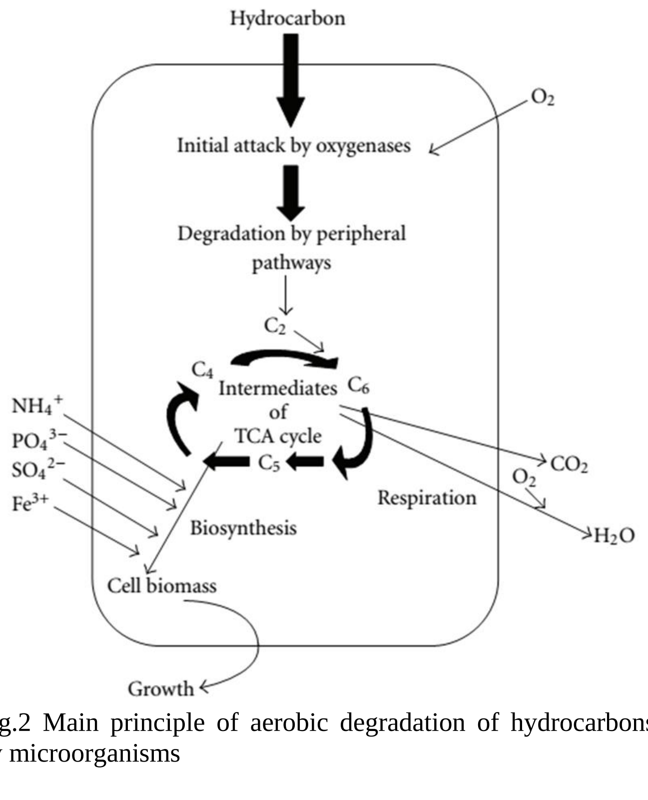

Fig.2 Main principle of aerobic degradation of hydrocarbons by microorganisms

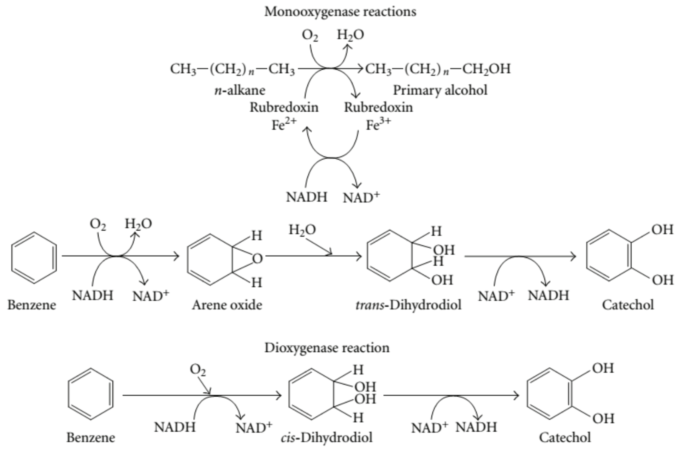

Fig .3 Enzymatic reactions involved in the processes of hydrocarbons degradation

The rapid and complete degradation of most organic pollutants occurs under aerobic conditions. Fig.2 illustrated the main principle of aerobic degradation of hydrocarbons [53]. The first intra-cellular organic pollutants attack takes the form of oxidation and activation and also the integration of oxygen is the enzymatic key a reaction catalyst via peroxidases and oxygenates. Pathways of peripheral degradation convert organic pollutants one step at a time into intermediates of the central intermediary metabolism, for example, tricarboxylic acid cycle. The cell biomass biosynthesis occurs from the metabolites of the central precursor such as acetyl-CoA, succinate, pyruvate, the saccharides necessary for various biosynthesis and growth are synthesized via gluconeogenesis. The PHs degradation could via a specific enzyme system. Fig.3 shows the first attack on xenobiotics by oxygenases. Other mechanisms are involved, such as microbial cells attached to substrates and biosurfactants production [16]. The mechanism of uptake associated with the attachment of the cell to the oil droplet is still unknown, but the biosurfactants production has been studied.

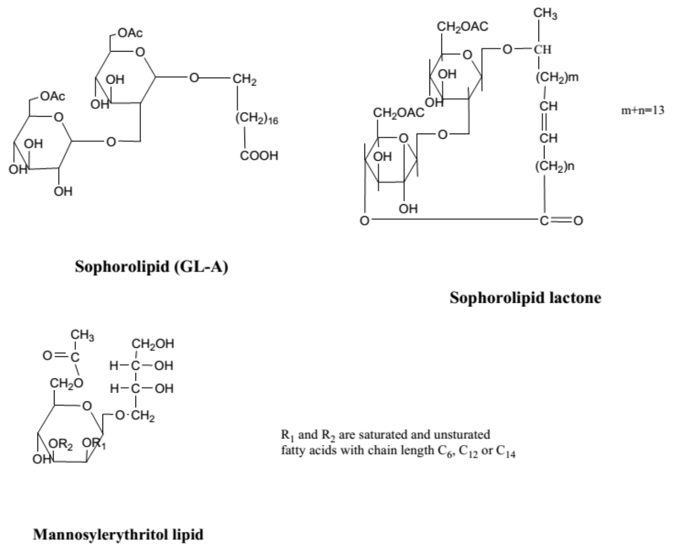

Fig 4 structural of sophorolipids

5. Hydrocarbons uptake using Biosurfactants

Biosurfactants are a heterogeneous group of surface active chemical compounds produced by a wide range of microorganisms, which are enhanced solubilization and contaminants removal [16, 54]. Biodegradation is enhanced with surfactants due to increased bioavailability of pollutants. Cameotra and Singh (2008) reported that the Pseudomonas aeruginosa and Rhodococcus erythropolis isolated from soil contaminated with oily sludge exhibited a higher ability to produce the biosurfactants and them able to degrade 90% of PH within six weeks in liquid media [55]. The biosurfactant used by a member of the consortium was produced and identified as a mixture of 11 rhamnolipid congeners, Within five weeks the consortium was degraded 91% of the hydrocarbon content in contaminated soil by 1% crude oil sludge [16]. The separate use of an additive along with the consortium brought up to 91-95% decrease of the hydrocarbon content within four weeks, with the crude biosurfactant preparation being a more effective enhancer of degradation. However, estimated by 98% depletion of hydrocarbon was achieved when both additives were added together with the consortium. Pseudomonad is the best-known bacteria capable of using PHs as sole carbon and energy sources and biosurfactants production [16, 55] . Besides bacteria, fungi play an important role in the producing Biosurfactants. Among fungi, *Candida ishiwadae*, *C. batistae*, *C. bombicola*, *Aspergillus ustus*, *Trichosporon ashii* and *Ustilago maydis* are the explored ones [56-59]. Numerous of these are known to produce biosurfactant on low-cost raw materials. The main type of biosurfactants produced by these strains is a sophorolipid (glycolipids). The sophorolipids structure produced by *Candida batistae* , *C. bombicola* and C. sp. SY16 are shown in Fig. 3 [57, 60, 61]. *Polisporum and Penicillium* can grow on nutrient agar medium with crude oil heavy fractions. *Mortierella sp. and Penicillium sp.* could utilize for conversion of crude oil to high molecular fractions [62]. The biosurfactants produce by *Aspergillus ustus* MSF3 and *Torulopsis bombicola* were used for the release of bitumen from the contaminated soil and degradation of hydrocarbons [58]. Mannosylerythritol lipids from *C. antarctica* have applications of potential in the biodegradation and removal of hydrocarbons in contaminated soil by oil and were also used to rinse oil and grease from the contaminated soil [63]. The Sophorolipids produced from *C. bombicola* and *C. lipolytica* are promising in the cleaning of oil tanks, microbial enhanced oil recovery, decontamination of polluted areas, industrial cleaning, house-hold applications and low-end consumer products [64].

6. Enzymes role in degradation of Hydrocarbons

Cytochrome P450 alkane hydroxylases constitute a superfamily of ubiquitous Heme-thiolate Monooxygenases, which is playing a significant role in the microbial degradation of oil, chlorinated hydrocarbons, and other compounds [65]. Cytochrome P450 enzyme systems were proved to be involved in the biodegradation of PHs. The ability of various yeast species to utilize n-alkanes and other aliphatic hydrocarbons as a sole source of carbon and energy is mediated by the existence of multiple microsomal Cytochrome P450 forms. These cytochrome P450 enzymes had isolated from species of yeast such as *Candida apicola*, *C. maltose* and *C. tropicalis* [66]. The alkane oxygenase systems diversity in prokaryotes and eukaryotes, which are actively participating in the degradation of alkanes with aerobic conditions 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 have been studied by Van Beilen and Funhoff [67]. Fungi are displayed as a powerful option for the degradation of PHs. They have several advantages over bacteria due to their ability to cultivate on a large group of substrates. At the same time, they produce extracellular hydrolytic enzymes, which can penetrate contaminated soil and remove pollutants [68] he rate and level of biodegradation of pollutants by fungi enzymes rely upon growth factors, such as, accessibility of nutrients, oxygen, and enzyme optimum conditions like temperature, pH, chemical structure of the compound, chemical partitioning in growth medium and cellular transport properties [29]. Ligninolytic fungi have been widely studied in the last few years because they produce extracellular enzymes with reduced substrate specificity. These evolved because of the irregular structure of lignin but resulted in the efficiency to also degrade and mineralize different organic pollutants. Fungal lignin peroxidases oxidize several of petroleum hydrocarbons directly, while fungal manganese peroxidases co-oxidize them indirectly via enzyme-mediated lignin peroxidation. Novotny et al. (2004), studied the enzymatic activities of LiP, MnP and laccase, and degradation by different species of ligninolytic fungi cultivated in liquid and soil. They exhibited that the degradation of anthracene and pyrene by *Phanerochaete chrysosporium*, *Pleurotus ostreatus* and *Trametes versicolor* depends on the MnP and laccase levels secreted into the soil. Thus, fungal degradation of PAHs is not as effective or fast as bacteria, but they are extremely non-specific and have the ability to hydroxylate a large variety of xenobiotics [69]. Furthermore, numerous fungi are naturally living on soil wastes and can be grown in soil and propagated during a solid matrix to remove petroleum hydrocarbons. These criteria make the environmental role of ligninolytic fungi in bioremediation. In addition to LiP, MnP, and laccase other fungal enzymes, like Cytochrome P450 monooxygenase, epoxide hydrolases, dioxygenases lipases and proteases have been widely studied for their ability to degrade PAHs [70].

CONCLUSION

Cleaning of PHs in the sub-surface environment is a real global problem. The biotic and abiotic factors play an important role in the biodegradation process of PH contaminated soils and water systems, such as physical conditions, nutrition, the ratios of various structural hydrocarbons present, the diversity of the microbial communities involved and the bioavailability of the substrate. Microbial degradation helps the elimination of spilled oil from the environment after removing large quantities of oil through various physical and chemical methods. In fact that possible because microorganisms have enzyme systems to degrade and use various hydrocarbons as a source of carbon and energy. Therefore, based on a current review, it can be concluded that microbial degradation can be considered as a key element of the cleaning strategy to PH remediation.

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