*Review Article*

**Microbial Degradation of Petroleum Hydrocarbons: an overview**

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1. INTRODUCTION

Oil Pollution is one of the major Environmental hazards which damage our ecosystem (1). Oil spills accidents are common phenomena and makes adverse effects on our ecological and biological systems (2). Major factors contributing to oil spills and hydrocarbon pollution in ecosystem are Industrial waste and oil accidents which happens during transportation and processing of Petroleum Products and Improper manual handling of equipments during mining(3,4). There are many adverse effects of Oil spills and hydrocarbon Pollution in environment which are carcinogenic, mutagenic and tetragenic effects (5). There are many strategies which are adapted for hydrocarbon remediation such as phytoremediation and bioremediation which are more successful Technologies due to their environment friendly and cost effective features (6). Petroleum hydrocarbons are among the many organic pollutants which are bounded to sea, estuaries and oceans (7).  Many organic and inorganic compounds can be present with petroleum hydrocarbons (8). When petroleum pollution contaminate environment the ratio of hydrocarbon degrading microorganism generally increases, so that increase in microbial degradation level will reflect the rate of Petroleum contamination (9, 10, 11). Microorganisms are said to use petroleum as their energy source (12). Bioremediation is a process used to treat contaminated area, including water, soil by altering environmental condition to stimulate growth of micro organism and degrade the target pollutants (13, 14). Hydrocarbon degrading microorganisms have a long list of genera including bacillus, Brevi Bacillus, pseudomonas, acetobacter, dietzia, Mithylobacterium and rhodococcus. (15, 16,17,18). From this point, Bioremediation is a very opted and suitable option for microbial contamination (19). This paper provides updated information on microbial degradation of petroleum hydrocarbon contaminants towards the better understanding in bioremediation challenges.

2. Petroleum Hydrocarbons: an overview

**Petroleum:**

Petroleum or fossil fuel is created from the incomplete biological decomposition of the naturally occurring organic debris of ancient bacteria and micro-organisms, primarily plankton and simple plants, buried deep within the Earth during geological time. These organic sediments were slowly decayed under anaerobic conditions in which some physical, chemical and biological processes were present due to high temperatures and pressure to produce oil. (22)

Oil and water were pushed out as rocks were compacted and migrated slowly to porous reservoir rocks, mostly sandstone or calcareous. Lastly, secondary migration took place inside the reservoir as the oil merged into a pool, usually covered by impermeable strata and often linked with natural gas. Petroleum formation needs lengthy periods of millions of years .But the composition and physical characteristics of petroleum from separate  reservoirs vary extensively. (12)

Because the initial decayed organisms contained components other than carbon and hydrogen, almost all crude oils and petroleum products are complicated mixtures. For instance, over 300 distinct hydrocarbons can be found in jet fuels. There are many indeterminate structures in these hydrocarbons. (3)

**Petroleum Exploration:**

It is stated that the world's first oil discovery began as soon as 20,000 B.C. Light lamps produced of wood by the earliest Mesopotamian civilizations. Apparently, the Chinese began around 5,000 B.C when they discovered petroleum and used it in drugs, waterproofing and warfare. Then, 3,000 B.C in it became common use of petroleum. (33)

In 1859, Colonel Edwin Drake, a railway driver, drilled the world's first oil well in Titusville, western Pennsylvania. Since then, the sector now provides approximately half of the world's energy, as well as petrochemical raw materials. The main oil-producing areas in the globe are the Persian Gulf, the United States, the USSR, North and West Africa, Mexico, Indonesia and Venezuela. (6)

In 1972, when they found offshore oil fields in Sabah, Sarawak and Terengganu, Malaysia began the petroleum industry. Shell in Miri, Sarawak, drilled the first oil well in Malaysia. Since then, the sector has made a significant contribution to the Malaysian economy, especially in these three countries. This resulted in Malaysia's domestic oil business, the Petroleum National Board (PETRONAS) being formed. (9)

Petroleum is a complex mixture of hydrocarbon and non hydrocarbons (12). Petroleum and its hydrocarbons are comprised of many different compounds which are distributed into 4 fractions:  aliphatic, aromatics, Nitrogen Oxygen sulphur (NOS) compounds and asphaltenes (20). Origination of Hydrocarbons starts from the biosynthetic activity of microorganisms and plants (21).  Petroleum or crude oil are comprised of variety of simple and complex hydrocarbons which are degraded by several microorganisms and each can break down a specific combination of molecules (23,24). Crude oil can be categorised into light, medium and heavy based on the ratio of heavy molecular weight constituent present in it (25).

1. **Aliphatics (Saturates):** They are bonded hydrocarbons and they are represented as highest percentage contribution in crude oil constituents. They are divided according to their chemical structure (26).
2. **Aromatics:**  They have one or many aromatic ring substituted with different alkyl groups (27).
3. **Nitrogen Oxygen sulphur (NOS Compunds):**  also known as resins compounds.  They contain non- hydrocarbon polar compounds, they have Complex structure and contain many Nitrogen Oxygen and sulphur atoms (28, 29).
4. **Asphaltenes:** They have Complex structure and they had greater molar mass as compared to other constituents therefore they are the innermost portion of the hydrocarbon structure (25, 30)

Hydrocarbons are equally distributed from surface of the environment to the deep subsurface by going through shallow sub surfaces. Due to this distribution there is change in Environmental condition happens where degradation of hydrocarbon occurs (31).

Oil contamination is highly hazardous to the environment. It has severe impacts in the plant as well as animal ecosystem including human health (37, 38). Research articles written by many researchers (10,12,14,25) enumerated the following as the effects of oil contamination on Fresh water and Marine Eco-system and ecological habitat:

1. A big proportion of the oil spill is distributed across the surface of the stagnant aquatic system resulting in the water below the surface being anaerobic. This results in the death of the natural flora and wildlife, where oxygen is the main component in their breathing.

2. Changes in natural environments physical and chemical.

3. Physical smothering effect and lethal or sub-lethal toxic effects on the aquatic life.

4. Aquatic birds have hypothermia, drowning, flight loss, poisoning, etc.

5. Exposure to crude oil may cause harm to the aquatic birds and animals ' lungs, liver, kidneys, intestines and other internal organs.

6. Impairment of reproduction in birds, fish and reptiles.

7. An oil-covered plant that cannot photosynthesize.

8. Oil spill considerably reduces invertebrate density and at least 5 km downstream taxonomic wealth.

9. Some fish species have been discovered impregnated with tarlike material with modified tracheal gills.

10. A big proportion of the oil spill, together with the sea shore, is emulsified and solidified, attached to sand, rock and stone.

11. Oil-related changes in the marine ecosystem effects on key organisms e.g. increased abundance of intertidal algae following the death of limpets, which normally eat the algae.

Other researchers (55,57,60,63) also enumerated the effects of oil contamination on human health. Such are:

1. Long-term exposure to contamination with crude oil leads to serious human and other animal diseases.

2. High-health hazards such as skin erythema (reddening), skin cancer, sinonasal cancer, gastrointestinal cancer, and kidney cancer are among the main elements found in crude oil.

3. Hydrocarbon vapor inhalation creates headache, nausea, swelling,respiratory irritation.

4. Benzene, toluene, ethylbenzene and xylene (BTEX), the main components of crude oil, cause mutations, diseases, birth defects, endocrine disorders, nervous disorders and liver illness, cancer, CNS impact, anxiety, irregular heartbeats.

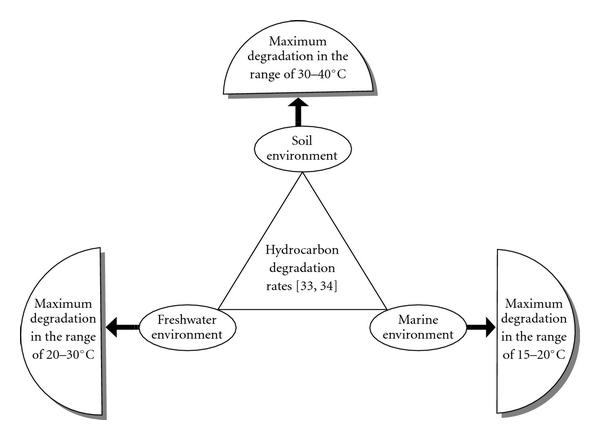
3. Microbial Degradation of Petroleum Hydrocarbons

Petroleum is the mixture of Hydrocarbons and other organic compounds which comprised of some organometallic constituents.  Petroleum products which are extracted from different oil reservoirs very differently in there composition and physical properties (32, 33). Microbial degradation of Petroleum hydrocarbons modifies crude oil with waxy properties into the beneficial ways but for the isolation of petroleum and its products requires use of thermophiles with heat stable enzymes. Bacteria are the most active agents in petroleum degradation,and they work as primary degraders of spilled oil in environment (34,35). Many studies have been done to identify measures to degrade petroleum hydrocarbons by use of microbes. Biodegradation and biotransformation and needed for cleanup of environment Microbial degradation is the major and ultimate natural mechanism by which one can cleanup the petroleum hydrocarbon pollutants from the environment Hydrocarbons in the environment are biodegraded primarily by bacteria, yeast, and fungi. The reported efficiency of biodegradation ranged from 6% to 82% for soil fungi, 0.13% to 50% for soil bacteria, and 0.003% to 100% for marine bacteria. Many scientists reported that mixed populations with overall broad enzymatic capacities are required to degrade complex mixtures of hydrocarbons such as crude oil in soil (33), fresh water (34), and marine environments (35, 36,37). These measures become Central topic for Petroleum microbiology. Though algae and protozoa are the important members of the microbial community in both aquatic and terrestrial ecosystems, reports are scanty regarding their involvement in hydrocarbon biodegradation. (38).

Microbial degradation is the main and ultimate natural process for cleaning up pollutants from the atmosphere from oil hydrocarbons. It is recorded the identification of biodegraded aromatic hydrocarbons derived from petroleum in marine sediments [27]. They investigated the comprehensive biodegradation of alkyl aromatics in marine sediments that took place before detection. Biodegradation of the n-alkane profile of crude oil and microorganisms (Arthrobacter, Burkholderia, Mycobacterium, Pseudomonas, Sphingomonas and Rhodococcus) for alkylaromatic degradation has been discovered to be involved.[28)

Environmental hydrocarbons are mainly biodegraded by bacteria, yeast, and fungi. The reported biodegradation efficiency ranged from 6%[29] to 82%[30] for soil fungi, from 0.13%[29] to 50%[30] for soil bacteria, and from 0.003%[31] to 100%[32] for marine bacteria. Many researchers have noted the need for blended populations with wide enzymatic capabilities to degrade complicated mixtures of hydrocarbons such as crude oil in soil [[33](https://www.hindawi.com/journals/btri/2011/941810/" \l "B33)], fresh water [[34](https://www.hindawi.com/journals/btri/2011/941810/" \l "B34)], and marine environments [[35](https://www.hindawi.com/journals/btri/2011/941810/" \l "B35), [36](https://www.hindawi.com/journals/btri/2011/941810/" \l "B36)].

Bacteria are the most effective agents in the degradation of petroleum and operate in the setting as main degraders of spilled oil[ 37, 38]. It is even known that several bacteria feed solely on hydrocarbons[39]. [36] mentioned 25 genera of degrading hydrocarbons and 25 genera of degrading hydrocarbons which were isolated from marine environment. There were 22 genera of bacteria and 31 genera of fungi in a comparable compilation by many researchers [33]. The extent to which bacteria, yeast, and filamentous fungi engage in the biodegradation of petroleum hydrocarbons has been the topic of restricted research in previous days, but appears to be a function of the ecosystem and local environmental conditions[7].



**Figure 1: Hydrocarbon degradation rates in soil, fresh water, and marine environments(81)**

4. Environmental factors affecting biodegradation of hydrocarbons:

It has been acknowledged that a number of limiting factors influence the biodegradation of petroleum hydrocarbons [53]. The petroleum hydrocarbon pollutant's structure and intrinsic biodegradability is the first and foremost consideration when assessing the suitability of a remediation strategy. Temperature plays a role among physical variable an important role in biodegradation of hydrocarbons by directly affecting the chemistry of the pollutants as well as affecting the physiology and diversity of the microbial flora. Atlas [52] found that at low temperatures, the viscosity of the oil increased, while the volatility of the toxic low molecular weight hydrocarbons were reduced, delaying the onset of biodegradation.

(1) **Chemical composition of hydrocarbons**- Hydrocarbons divided into four major classes the saturates, the aromatics, the asphaltenes, and the raisins (39). Rate of biodegradation is high in case of saturates and in case of aromatics, asphaltenes and raisins biodegradation rate is low (40).

(2) **Physical composition-** oil spill in water causes dispersion of hydrocarbon occurs which form oil in water emulsion (41) which increases surface area of oil on water surface and it makes microbes to attack on surface (42). The tarballs which are large aggregates of undegraded oil restrict access by micro organisms because of their Limited surface area (43).  The formation of emulsions through microbial production and release of biosurfactants is important process in the uptake of hydrocarbons by bacteria and fungi (44)

(3) **Temperature-** There is major effect of temperature on the petroleum biodegradation process. It affects physical nature, composition of oil. It influences the rate at which microorganism degrading hydrocarbons and composition of microbes and their community (45).  When temperature decreases it results also in decreasing rate of degradation and rate of degradation increases with increase in temperature depending on composition of Hydrocarbons of a petroleum mixture (46, 47).

(4) **Nutrients**- Release of hydrocarbon in aquatic environment which contains low concentration of inorganic nutrients which produces excessive high carbon, nitrogen ratio which was not suitable for microbial growth (45, 48). Many oil spills fate concluded in a way that concentration of Nitrogen and phosphorus limited with respect to rates of hydrocarbon biodegradation (45). Many studies indicates that concentration of Nitrogen and phosphorus can limit extent of hydrocarbon degradation after oil spills. Fertilizers which contain nitrogen and phosphorus can be used to stimulate microbial hydrocarbon degradation (49).

(5) **Oxygen**- Aerobic condition are necessary for microbial oxidation of Hydrocarbons in environment (50).  Availability of oxygen is mostly depends on the variety of soils, rate of microbial oxygen consumption and the presence of utilizable substrate which can cause oxygen depletion (51). Rates of hydrocarbon degradation decrease with decreasing oxygen reduction potential that is with increasing anaerobiosis (52). Oxygen plays important role in hydrocarbon degradation. Major degradative Pathways for saturated and aromatic hydrocarbon involved oxygenase and Molecular oxygen (53, 54).

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5. Mechanism of Petroleum Hydrocarbon Degradation:

Petroleum and its products contain a category of molecules referred as hopanoids that are ordinary found in microorganism’s cell walls. Many researchers indicated that these fuse at some point originated a minimum of partially from micro organisms and biodegradation of these fuels has perpetually been occurring to some extent (55). Biodegradation of Hydrocarbons is by natural population of microorganism give conversion of harmful substances into forms that are less or non virulent and the present one among the first mechanism to be used for oil and diesel degradation in expensively (52). When oil is filled within the ocean and seas it undergoes changes with the passage of time which brought by wave action the light and inherent microbial community (56).  A huge quantity of oil hydrocarbon enters in surrounding atmospheric accidentally or because of human activities causing a major drawback within the world because of this toxicity and harmful properties to surroundings (57).

Biostimulation has been widely studied in the main place application wherever it is able to look at changes within the microbic patterns of the community.

Bioaugmentation could be a technique that improves the degrading potential of contaminated areas through the introduction of specific microbic strains or consortium which can degrade economically (58). Micro organisms play a very important role in the fate of soil contamination in surrounding environment by working with Bioadsorption and biodegradation (59). In the method of bioremediation microorganisms works as biosorbent which accommodates organic pollutants and as a bioreactor that degrades pollutants (60).

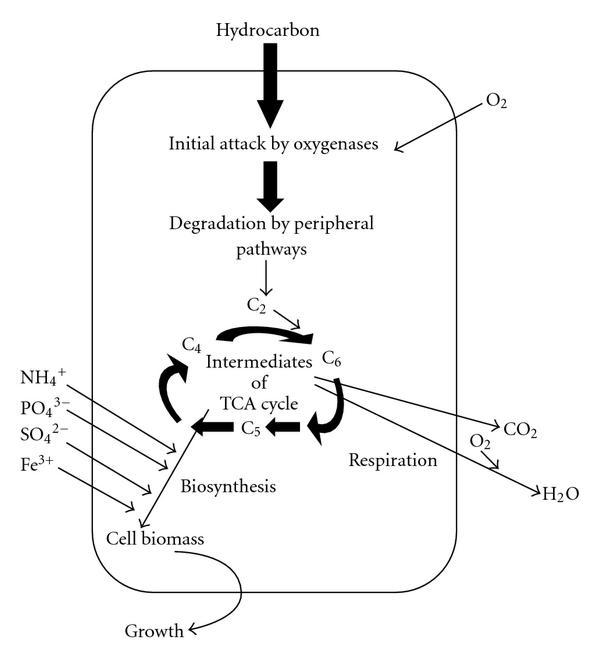
6. Pathways for Biodegradation of Petroleum Hydrocarbon pollutants:

Under aerobic circumstances, the fastest and most complete degradation of most organic pollutants occurs. Figure 2 illustrates the primary principle of hydrocarbon aerobic degradation [82]. The original intracellular assault of organic pollutants is an oxidative method and the enzymatic main response catalyzed by oxygenases and peroxides is the activation and integration of oxygen. Peripheral degradation pathways transform organic pollutants step by step, such as the tricarboxylic acid cycle, into intermediates of the central intermediate metabolism. Cell biomass biosynthesis happens from metabolites of the key precursor, such as acetyl-CoA, succinate, pyruvate. Gluconeogenesis synthesizes the sugars needed for different biosyntheses and development (43).

Micro organisms can utilise petroleum hydrocarbon in two ways that is they can degrade hydrocarbon to obtain energy or assimilate them into cell Biomass (10). There are two ways for addition of Petroleum hydrocarbons (10, 26,61).

1. Aerobic
2. Anaerobic

Hydrocarbons degradation is a aerobic process but many micro organisms are said to be involved in anaerobic degradation of hydrocarbons (26, 27).

Aerobic and Anaerobic Pathways of microbial degradation petroleum hydrocarbon pollutants had common reaction that is oxidation, reduction, hydroxylation, and dehydrogenation (26,61). Biodegradation can be characterized as increase in relative abundance of Polar fractions as well as of saturated and aromatic hydrocarbons (62,63) **Figure 2: Main principle of aerobic degradation of hydrocarbons by microorganisms.**

Specific enzyme systems can mediate the degradation of oil hydrocarbons [75]. Other processes engaged are

(1) Microbial cell attachment to substrates

(2) Bio surfactant production[76].

The absorption mechanism associated with cell attachment to petroleum droplets is still unknown.(24)

7. Phytoremediation and Bioremediation of Petroleum hydrocarbons:

Bioremediation is the most effective and widely used technique for treating petroleum hydrocarbon pollution in aquatic and Terrestrial ecosystems (63,64) This is an environmental friendly method which was regularly getting popular due to its potential to clean up oil contaminated area (65).It can be defined as the use of biologically mediated processes to detoxify, degrade or transform pollutants to an refined state(66). This is a popular and hands on method for degrading Petroleum hydrocarbons as it is simple method to maintain, cost effective and can be used to large areas (67). There is another method used for remediation of Petroleum hydrocarbons which is known as phytoremediation which can be defined as the use of plants and their related micro organisms to metabolise, transform, assimilate and degrade toxic or hazardous compound present in soil, water due to oil refineries(68,69,70). Phytoremediation is a environment friendly, effective and low cost method (68). This is an economic method for waste management, removal of hydrocarbon toxic in nature, dangerous organic materials and nutrients (71).  Remediation of Petroleum polluted soils biological techniques not only would create a healthy environment but also restore soil function (72). Bioremediation method includes bioaugmentation and biostimulation.  Bioaugmentation means to inoculating micro organism into the contaminated soil to degrade hydrocarbons .Biostimulation implies supplying nutrients to the soil to stimulate the hydrocarbon degrading capacity of indigenous micro organism (73).

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| **Advantages** | **Disadvantages** |
| Relatively low cost | Longer remediation times |
| Easily implemented and  Maintained | Climate dependent |
| Several mechanisms for  removal | Effects to food web might be  unknown |
| Environmentally friendly | Ultimate contaminant fates  might be unknown |
| Aesthetically pleasing | Results are variable |
| Reduces landfilled wastes |  |
| Harvestable plant material |  |

**Table 1: Advantages and disadvantages of phytoremediation over**

**Traditional technologies.(83)**

Research and application of phytoremediation for the treatment of petroleum hydrocarbon contamination over the past fifteen years have provided much useful information that can be used to design effective remediation systems and drive further improvement and innovation (74). Many contaminated sites could be remediated by phytoremediation. However, little is understood about contaminant destiny and mechanisms of conversion (75). There is little data about contaminant extraction rates and efficiencies that are directly attributable to plants under field circumstances. Microbial degradation in the rhizosphere could be the most important mechanism for removing organic diesel ranges in contaminated vegetated soils. This is due to extremely hydrophobic contaminants such as PAHs and their self-absorption to soil decreases their bioavailability for plant uptake and phytotransformation (76). Genetically engineered microorganism (GEM) applications in bioremediation have gained much attention to enhance the degradation of hazardous waste under laboratory circumstances. There are reports of various bacteria degrading environmental pollutants. Higher degrading ability was shown by the genetically engineered bacteria. Ecological and environmental concerns and regulatory limitations, however, are significant barriers to GEM testing in the sector. Before GEM can provide an efficient clean-up method at a reduced price, these issues need to be solved (77).

**8. Enzymes Participating in Degradation of Hydrocarbons**

Cytochrome P450 alkane hydroxylases are a super family of ubiquitous heme-thiolate monooxygenases that play an significant part in oil microbial degradation, chlorinated hydrocarbons, fuel additives, and many other compounds (74). Enzyme systems are needed to bring oxygen into the substratum to start biodegradation, depending on the chain length.. Higher eukaryotes generally contain several different P450 families that consist of large number of individual P450 forms that may contribute as an ensemble of isoforms to the metabolic conversion of given substrate. In microorganisms such P450 multiplicity can only be found in few species (75). Cytochrome P450 enzyme systems was found to be involved in biodegradation of petroleum hydrocarbons. The capability of several yeast species to use 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 been isolated fromyeast species such as *Candidamaltosa*, *Candida tropicalis*, and *Candida apicola* (78). The diversity of alkaneoxygenase systems in prokaryotes and eukaryotes that are actively participating in the degradation of alkanes under aerobic conditions like Cytochrome P450 enzymes, integral membrane di-iron alkane hydroxylases (e.g., *alkB*), soluble di-iron methane monooxygenases, and membranebound copper containing methane monooxygenases have been discussed by Van Beilen and Funhoff (77).

#### 10. Genetically Modified Bacteria

Genetically engineered microorganism (GEM) applications in bioremediation have gained much attention to enhance the degradation of hazardous waste under laboratory circumstances. There are reports of various bacteria degrading environmental pollutants. There are many examples of the appropriate use of genetic engineering technology to enhance hydrogen bioremediation using bacterias (77) Higher degrading ability was shown by the genetically engineered bacteria. Ecological and environmental concerns and regulatory limitations, however, are significant barriers to GEM testing in the sector. Before GEM can provide an efficient clean-up method at a reduced price, these issues need to be solved(79).The use of genetically engineered bacteria has been applied to controlling the process of bioremediation, tracking strain, reaction to stress, endpoint analysis, and evaluation of toxicity. (80). Chlorinated compounds, aromatic hydrocarbons, and nonpolar toxicants were included in the range of contaminants tested. Combining understanding of microbiology and ecology,biochemical mechanisms, and field engineering designs are essential elements for successful in situ bioremediation using genetically modified bacteria.(81)

11**. Conclusion**

Cleaning up of petroleum hydrocarbons in the subsurface environment is a real world problem. A better understanding of the mechanism of biodegradation has a high ecological significance that depends on the indigenous microorganisms to transform or mineralize the organic contaminants. Microbial degradation process aids the elimination of spilled oil from the environment after critical removal of large amounts of the oil by various physical and chemical methods. This is possible because microorganisms have enzyme systems to degrade and utilize different hydrocarbons as a source of carbon and energy. Therefore, based on the present review, it may be concluded that microbial degradation can be considered as a key component in the cleanup strategy for petroleum hydrocarbon remediation. Petroleum hydrocarbon pollutants are priority pollutants as they are resistant to degradation due to their low reactivity. These persistent organic pollutants (POPs) pose serious threat to the human and environmental health. Bioremediation is recognized as an efficient, economic and versatile alternative to physicochemical treatment. Biodegradation of this pollutant can be performed using oleophilic microorganisms either as individual organism or consortium of microorganisms to control environmental pollution. Understanding factors affecting biodegradation is of great research interest. Catabolic pathways involved in biodegradation (aerobic/anaerobic) show the way to design efficient strategies for bioremediation of petroleum hydrocarbon pollutants impacted environments.

**10.References:**

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