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A Review on Environmental Contamination of Petroleum Hydrocarbons and its Biodegradation

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Abstract

Petroleum hydrocarbon contamination is one of the major environmental problems resulting from its large scale uses in transportation, industrial, agricultural and other sectors. Accidental releases and workshop seepage of petroleum products are of key concern for the environment. A variety of petroleum hydrocarbons such as crude oil, diesel, gasoline, heavy oil, kerosene etc. are used extensively as energy source, although their contaminations in soil and water have adverse effects. Contamination of soil with petroleum products deteriorates soil's biochemical and physicochemical properties; it also limits the growth and develop¬ment of plants. Oil spills has devastating effects on marine ecosystems, it hindered oxygen penetration in water which affect marine ecosystem. Petroleum hydrocarbon has several chronic and acute effects on human health. Inhalation, ingestion and dermal contact of these pollutants cause many harmful diseases. Bioremediation is the promising technology for the treatment of these petroleum hydrocarbons since it is cost-effective and environment friendly. Several microorganisms have the capability to grow on it, use it as a sole source of carbon and mineralize it into simpler forms in natural environment. This review provides an overview on the effects of petroleum hydrocarbons on soil, water and human health and degradation of these petroleum hydrocarbons using microorganisms.

Keywords: Petroleum hydrocarbon; Oil spills; Contamination; Adverse effects; Biodegradation

Introduction

Petroleum hydrocarbons are extensively used worldwide as a fuel. Because of its huge demand as an energy source, contamination occurs quite often as a result of exploration, production, maintenance, transportation, storage and accidental release, leading to significant ecological impacts. As the modern civilization developed, it creates pressure on the energy source, especially on petroleum hydrocarbons [1]. Approximately 5.74 million tonnes of oil were lost as a result of tanker incidents from 1970 to 2014 [2]. Workshop seepage is also another source of petroleum hydrocarbon contamination. The presence of various kinds of automobile and machinery vehicles has caused an increase in the use of motor oil. Used motor oils spillage such as diesels or jet fuels contaminate natural environment [3].

Environmental contamination by petroleum hydrocarbon is one of the significant concerns of recent world. It has disastrous and catastrophic consequences, not only on the human beings but also on other biotic components of the ecosystem [4]. When an oil spill occurs, oil floats being less dense than water. It also pollutes air since the most volatile hydrocarbons start to evaporate initially after the oil spills [5]. There are different types of physical and chemical method for the remediation of oil contaminated soil such as burying, evaporation, dispersion, washing etc. Soil vapor extraction, soil washing and incineration are some of the mechanical methods. However, these technologies are expensive and can lead to incomplete decomposition of contaminants [6]. There are also some chemical methods but these are very costly approach to treat oil contaminated sites. Therefore, it is important to develop an innovative, low cost and eco-friendly method for the removal of hydrocarbon contamination from the soil. Bioremediation method is considered to be more economical and safe method for the treatment of hydrocarbon contaminated site [7]. Several microorganisms have the ability to grow on hydrocarbon contaminated soil and they are capable to degrade oil than those microorganisms which grow on non-contaminated sites of oil [8]. This paper provides information on the effects of petroleum hydrocarbon on soil, water and human health and degradation of these petroleum hydrocarbon using microorganisms.

Petroleum Hydrocarbons

Crude Oil

Crude-oils are mainly short-chain hydrocarbons [9], it is composed of complex mixtures of paraffinic, alicyclic and

aromatic hydrocarbons and a smaller proportion of non-hydrocarbon compounds such as naphthenic acids, phenols, thiol, heterocyclic nitrogen, sulphur compounds as well as metallo-prophyrins and asphaltenes [10]. Crude oil as a complex mixture is produced by incomplete decomposition of plant and animal biomass over a long time [11]. The carbon content normally is in the range 83-87%, and the hydrogen content varies between 10 and 14%. In addition, varying small amounts of nitrogen, oxygen, sulfur and metals (Ni and V) are found in crude oils [12].

Diesel

Diesel fuels are middle distillates of crude petroleum separated by fractional distillation. Other middle distillates include kerosene and aviation fuel. The carbon number of diesel oil hydrocarbons is between 11 and 25 and the distillation range is between 180 to 380°C. Diesel oil contains 2000 to 4000 hydrocarbons, which cannot be totally separated by gas chromatography [13]. It includes approximately 64% aliphatic hydrocarbons, l-2% olefinic hydrocarbons and 35% aromatic hydrocarbons [14]. It composed of four main structural classes of hydrocarbons [15].

a) n-alkanes or n-paraffins (linear saturated hydrocarbons).

b) isoalkanes or isoparaffins (branched saturated hydrocarbons).

c) cycloalkanes or naphthenes (saturated cyclic alkanes).

d) Aromatics.

Gasoline

Gasoline is a generic term used to describe volatile, inflammable petroleum fuels used primarily in internal combustion engines to power passenger cars and other types of vehic1e, such as buses, trucks, motorbikes and aircraft [16]. In gasoline composition, aromatics amount to about 50% of the total hydrocarbon content. Iso-alkanes amount to about 35% alkanes, alkenes and cycloalkanes are present in minor quantities. Its distillation range is from 30-35°C to 180-200°C [17]. It is a complex mixture of volatile hydrocarbon compounds with a nominal boiling-point range of 50-200°C (USA) or 25-220°C (Europe) for automotive gasoline. Hydrocarbons are predominantly in the C4-C12 range [18]. Gasoline is very flammable; it catches on fire quite easily, evaporates quickly, and forms explosive mixtures with air. Most people can begin to smell gasoline at 0.25 parts of gasoline per million parts of air (ppm). Gasoline does not dissolve readily in water.

Heavy Oil

Heavy oils are naturally occurring materials which contain hydrocarbons that are synthesized by living organism usually account for less than 20% by weight of the petroleum and petroleum like materials. It is the residue of crude oil distillation and its composition is carbon 88%, hydrogen 10%, sulfur 1%, H2O 0.5%, ash 0.1% by weight, and may contain dispersed solid or semi-solid particles (asphaltenes, minerals and other leftovers from the oil source, metallic particles from the refinery equipment, and some dumped chemical wastes), plus some 0.5% water. It leaves a carbonaceous residue in the tanks, and may have up to 5% of sulfur [19]. The limited constituents of heavy oil that dissolve in water become available for biodegradation when release to the environment. Heavy oils are not readily biodegradable. Heavy oils are also mutagenic.

Kerosene

Kerosene is a liquid mixture of chemicals produced from the distillation of crude oil. Kerosene is a major component (>60%) of aviation (jet) fuels, is used for "oil" central heating systems and can be used as a cleaning agent or solvent [20]. Kerosene contains hydrocarbons C11 to C12. It is flammable and practically insoluble in water [21].

Petroleum Hydrocarbon Contamination

Though social and economic development largely depends on petroleum hydrocarbon as it is a dominant source of energy, it has caused a huge area of contamination and relevant adverse effects [22]. The contamination of petroleum hydrocarbon disseminate from soil, water to human health.

Petroleum Hydrocarbon Contamination on Soil

Petroleum hydrocarbon contamination of soil is a widespread global environmental concern. Oil and fuel spills in soil are among the most extensive and environmentally damaging pollution problems as it is threatening to human health and ecosystems, especially in cold region [23]. Biochemical and physicochemical properties of soil is deteriorated by refinery products and it also limits the growth and develop¬ment of plants [24]. Water and oxygen deficits as well as to shortage of available forms of nitrogen and phosphorus are the main changes of soil properties due to contamination with petroleum-derived substances [25]. Petroleum hydrocarbon contaminated soil causes organic pollution of underground water which restricts it use and causes economic loss, environmental problems and decreases the agricultural productivity of the soil. Microorganisms, plants, animals and humans are facing vulnerable situation because of the toxicity of petroleum hydrocarbons [26]. Soil enzymes are one of the important biotic components which are responsible for soil biochemical reactions. Petroleum hydrocarbon has adverse effects of on soil enzyme activities [27].

Effect of Crude Oil on Soil

Oil spills affect plants by creating conditions which make essential nutrients like nitrogen and oxygen needed for the plant growth unavailable to them [28]. Crude oil contamination at different levels caused significant reduction in the growth of

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the plant using plant height, fresh weight and leaf area and the effect is proportional to the levels of contamination [29]. Crude oil pollution has also adverse effects on soil fertility and plant production. It could reduce or stop plant growth leading to death as a result of forming a physical barrier and coating the roots [30]. Table 1 shows adverse effects of crude oil contaminated soil in different plant species.

Table 1: Adverse Effects of Crude Oil Contaminated Soil on Different Plant Species.

Adverse Effects	Plant	References
Increase in total carbohydrates, total proteins, amino acids and praline in shoot and a decrease in chlorophyll contents of the leaves of 85-days-old seedlings. Meanwhile, there were decreases in total carbohydrates in roots.	Jojoba (*Simmodsiachinensis*)	Al-Hawas et al. [7]
Crude oil pollution has an adverse effect on growth, yield and leaf chlorophyll content.	Air Potato (*DioscoreabulbiferaL.*)	Osuagwu et al. [58]
The plant growth was reduced significantly in low levels.	Horsetail tree (*Casuarinaequisetifolia*)	Ali et al. [8]
Hindered germination, reduced heights and girths were observed.	Abelmoschusesculentus	Oyedeji et al. [59]
Significant reductions in plant height, leaf area and stem diameter was observed.	Maize (*Zea mays L.*)	Agbogidi et al. [4]
Grain yield was significantly reduced at 95% level.	Maize (*Zea mays L.*)	Ekundayo et al. [24]
A significant reduction in heights of seedlings, leaf length and number of leaves.	Soybean (*Glycine max*)	Ekpo et al. [23]
Root development is reduced.	Red beans (*Phaseolusnipponesis*) and corn (*Zea mays*)	Baek et al. [14]

Effect of Diesel on Soil

Diesel oil has a much stronger inhibitory effect on nitrification than petrol (Kucharski et al., 2010). Diesel oil can cause chronic or acute effects in the plants. Interference in the hydric relations of the plants is caused by diesel oil pollution [31]. Table 2 shows the adverse effects of diesel contaminated soil on different plant species.

Table 2: Adverse Effects of Diesel Contaminated Soil on Different Plant Species.

Adverse effects	Plant	References
Significantly affected the germination, GSI and seedling growth	*Schinusterebinthifolius*	Bona et al. [16]
Inhibition of germination and the longitudinal growth	*Festucaarundinacea*	Zarinkamar et al. [81]
Poses threats to the survival and development of plants	*Vignaunguiculata*	Njoku et al. [54]
Plant growth parameters were affected adversely	Eggplant (*Solanummelongena*)	Akujobi et al. [6]
Depressed yield of yellow lupine aboveground parts and roots	*Lupinusarboreus*	
	Wyszkowski et al. [80]	
Caused negative effect on the content of all macroelements in oat, favorable effect on the accumulation of most of macro-elements in maize	(*Avena sativa L.*) And Maize (*Zea mays L.*)	Wyszkowski et al. [79]
Reduced the number of germinating seeds and then limited their development.	Faba bean (*Viciafabassp. minor*) and rape (*Brassica napus var.*)	Hawrot-Paw et al. [28]
High contamination significantly reduced the germination and growth	*Zea mays, Vignaradiata, Sorghum vulgare* and *Pennisetumglaucum*	Luhach and Chaudhry [45]
Diesel oil rather than crude oil this species to be killed	*Dryusintegrz'joliu*	Walker et al. [77]
Reduction in the length of the radicle for the four crop plants	*Arachishypogaea, Vignaunguiculata, Sorghum bicolor* and *Zea mays*	Ogbo [56]

Petroleum Hydrocarbon Contamination on Water

Petroleum hydrocarbon released in to the sea, normally during transportation, leading to the pollution of several sites, and can eventually reach the coasts. Oil spills ranging from low-level discharges to catastrophic accidents threatened coastal environments; large spills commonly are followed by clean-up efforts, but complete containment is rare [32]. As solubility of petroleum hydrocarbon in water is generally low, certain fractions of it float in water and form thin surface films, which will facilitate agglomeration of particles and natural organic matter, and impact on oxygen transfer. Other heavier fractions will accumulate in the sediment at the bottom of the water, which may affect bottom-feeding fish and organisms [33].

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Effects of Petroleum Hydrocarbon on Human Health

Effects of Diesel on Human Health

Occupational exposure may potentially occur, during manual filling or discharge operations in petrochemical industry [34]. Occupational exposure to diesel oil has been associated with the following operations: manually handled filling and discharge; marine diesel bunkering involving the manual handling of discharge lines; retailing through filing stations; tank dipping, pipeline and pump repairs, filter cleaning in refineries, distribution terminals and depots; tank inspection, cleaning and repairing; manufacture, repair, servicing and testing of diesel engines or equipment and injection and fuel systems; routine sampling and laboratory handling of diesel oils; and practices in which diesel oils are used as cleaning agents or solvents [35]. Skin exposures may occur whilst refueling domestic vehicles and pulmonary exposure may result from aspiration of liquid during manual siphoning [36]. Table 3 represents acute effects of diesel on human health.

Table 3: Acute Effects of Diesel.

Type of Exposure	Acute Effects
Inhalation	a) Inhalation of diesel may cause headache, dizziness, drowsiness, in coordination and euphoria. b) Aspiration into the lungs causes pneumonitis with choking, coughing, wheeze, breathlessness, cyanosis and fever. c) Inhalation of diesel vapour may lead to CNS / respiratory depression and cardiac arrhythmias (Cocawe [20]).
Ingestion	The signs of toxicity following oral intake are generally stated to include nausea, vomiting, diarrhoea, irritation of the aero-digestive and Gastrointestinal tracts (Cocawe [20]).
Ocular Exposure	a) This product is expected to be pH neutral but may be irritating to the eyes causing an immediate stinging and burning sensation with lachrymation. b) Eye exposure to diesel may cause transient pain and/or hyperaemia (UK HPA [73]). c) Diesel is generally considered to be less irritating to the eyes than other middle distillate fuels such as kerosene or petrol (IPCS [31]).
Dermal Exposure	a) Exposure to diesel fuel through the skin and by inhalation may occur during its production, storage, distribution and use as well as during maintenance of diesel engines (IARC [30]). b) Acute dermal exposure may result in local irritation which is generally more severe than that seen with other middle distillate products (Kitschier, 1999). c) There may be transient pain with erythema, blistering and superficial burns (Cocawe [20]). d) Diesel will remove natural fat from the skin; repeated or prolonged exposure can result in drying and cracking, irritation and dermatitis. e) Some individuals may be especially susceptible to these effects. Excessive exposure under conditions of poor personnel hygiene may also lead to oil acne and folliculitis and with some products, development of warty growths may occur and these may become malignant subsequently (Cocawe [20]).
Neurotoxicity	It causes neurological disorders including drowsiness, neurasthenia and decreased sensorimotor speed (ATSDR [12]).
Nephrotoxicity	a) Several case studies have cited acute renal failure (secondary to acute renal tubular necrosis) as a potential complication following acute exposure to diesel. b) Signs included oliguria (progressing to anuria), nausea, abdominal cramps and diarrhea (UK HPA [73]).

Chronic Effects of Diesel

Prolonged skin exposure to diesel may cause a variety of dermatitic conditions and is generally a result of inadequate or inappropriate use of personal protective equipment. Also hyperkeratosis may be a common feature of regular contact with diesel [37].

a) Diesel does not have a measurable effect on human reproduction or development.

b) There is currently inadequate evidence to link diesel with the incidence of cancer in humans but there is limited evidence for carcinogenicity in animals following prolonged exposure [36].

Effects of Gasoline on Human Health

Gasoline has harmful effect on soil and water as well as human health. Inhaling or swallowing large amounts of gasoline can cause death [38]. Serious lung injury may occur if droplets of gasoline are inhaled (e.g. if vomiting occurs after ingestion). Inhalation may cause headache, dizziness and drowsiness. In some cases, sickness and diarrhea may occur. Gasoline vapour may be irritating to the eyes and lungs. Prolonged skin exposure to gasoline may cause a variety of skin conditions. Long-term exposure to high levels of gasoline is associated with a range of disorders affecting the nervous system [39].

Effects of Kerosene on Human Health

Kerosene is not particularly poisonous. However, if a child or adult accidentally swallows kerosene, medical advice should be obtained immediately as there is a small risk of short-term lung damage if vomiting occurs. Frequent skin exposure may lead to skin damage [36]. Kerosene possesses moderate to high acute toxicity to biota with product-specific toxicity related to the type and concentration of aromatic compounds. Kerosene spills

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could result in potential acute toxicity to some forms of aquatic life [40].

Bioremediation of Petroleum Hydrocarbon

Petroleum hydrocarbon contamination is highly hazardous to the environment. It has severe impacts on the plants as well as animal ecosystem including human health. Various conventional methods include physical and chemical technique which are costly and caused negative consequences [41]. In such cases, bioremediation is the most effective and it may be defined as any activity encouraging the natural process of degradation of petroleum hydrocarbon [42]. Bioremediation transforms the toxic substances to harmless products such as CO2, H2O and fatty acids [43].

Indigenous microbial communities have an important role in oil contaminant degradation. Once the site is contaminated, the microbial community composition will be greatly changed [44]. Microorganisms involved in the degradation of contaminant increase in their number till the contaminant is present. After the degradation of the contaminant the microbial population decreases itself naturally [45]. The rates of degradation of different classes of organic compounds in petroleum mixture vary widely. The biodegradation of n-alkanes is more rapid (except for the most volatile fraction C5-C9), followed by simple aromatics such as benzene, toluene and xylene-isoalkanes whereas cycloalkanes and aromatics degrade more slowly [46]. Hydrocarbons differ in their susceptibility to microbial attack. The susceptibility of hydrocarbons to microbial degradation can be generally ranked as follows: linear alkanes >branched alkanes >small aromatics >cyclic alkanes. Some compounds, such as the high molecular weight polycyclic aromatic hydrocarbons (PAHs), may not be degraded at all [47].

Most of the oil spillage occurs in the sea during transportation. Several studies have done by many scientists on microbial degradation of hydrocarbon in marine environment. Sutiknowati found that *Alcanivorax, Marinobacter* and *Prosthecochloris* are some hydrocarbon degrading bacteria which are found in marine environment [48]. According to Chikere et al. [49] *Bacillus, Nocardia, Staphylococcus, Pseudomonas, Flavobacterium, Escherichia, Acinetobacter* and *Enterobacter. Bacillus spp* are isolated from marine sediments of the Niger Delta and they can degrade hydrocarbon. *Bacillus spp* showed 92.5% degradation of hydrocarbon content during the spillage of Lubricating oil in the water, studied by Gopinath et al. [50] and Dhar et al. [51] studied on the biodegradation of petroleum hydrocarbon of ship breaking yard and found that *Fusariumm oniliforme* caused the maximum degradation of octane (58%) and diesel (56%), *Penicillium corylophilum* caused the same of kerosene (40%). Soil contamination also remedy by using microorganisms. In Kuwait, *Bacillus subtilis* strains are isolated from oil contaminated soil [52]. Crude oil is a complex mixture consisting of aliphatics, aromatics, resins and asphaltenes. It caused potential hazards for the environment. Several studies have been done for the biodegradation of crude oils. Some of them are given in Table 4.

Table 4: Biodegradation of Crude Oil by Various Microorganisms.

Degradation of crude oil	Organism	References
Maximum crude oil biodegradation of 78% was achieved	Consortium containing five cultures (*Micrococcus sp. Corynebacterium sp. Flavobacterium sp., Bacillus sp.* and *pseudomonas sp.*	Rahman et al. [64]
Increase in optical densities and utilize crude oil as a carbon and energy source.	*Bacillus spp.*	Sepahi et al. [67]
Maximal degradation (93%) of crude oil	The white rot fungus *Polyporus sp.*	Hadibarata and Tachibana [27]
Removed nearly 52.1% of crude oil	consortium of *Rhizobiales sp., Pseudomonas sp., Brucella sp., Bacillus sp., Rhodococcus sp., Microbacterium sp. and Roseomonassp*	Zhao et al. [82]
Potential biodegradation of in situand/or ex situ	Bacillussubtilis and PseudIomon s aeruginosa	Latha and Kalaivani [40]
Degrade long chain crude oil alkanes in a range between 46.64% and 87.68%.	Bacillus and GeobaciIllus species	Maintains et al. (2006)
mixed bacterial consortium decreased from 77 to 45% as the concentration of crude oil	Bacillussp, *Corynebacterium sp*, and *Pseudomonas sp*	Sathish kumar et al. [66]
At 1% crude oil concentration, the mixed bacterial consortium degraded a maximum of 78%	*Micrococcus sp., Corynebacterium sp., Flavobacterium sp, Bacillus sp.* and *Pseudomonas sp*	Rahman et al. [63]
Crude oil degrade at 58% and 40%	Acinetobacter calcoaceticus and Alcaligenesodorans	Lal and Khanna [39]

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Soil bacteria are capable of adapting to degrade environmental pollutants; some soil types may have indigenous bacteria that are naturally suitable for degradation. But High concentration of diesel can be toxic to microbes and inhibit degradation, so bacterial degradation is possible when the concentration of contaminant is below the threshold of toxicity [53-59]. Lawson studied on diesel utilizing bacteria on contaminated soil [60-65]. They found that six hydrocarbon utilizing bacterial genera, Bacillus, *Staphylococcus, Enterobacter, Yersinia, Proteus,* and *Alcaligenes* were present in the soil and the study clearly indicated that Ghanaian soils contain diverse bacterial genera capable of degrading and utilizing diesel oil as carbon source. Biodegradation of diesel oil was performed using a diesel oil-degrading bacterial consortium, in both laboratory and pilot scale experiments by Marquez-Rocha et al. [66]. The concentration of diesel in soil treated with the bacterial consortium was reduced to <15% of the initial concentration, within a period of five weeks in both laboratory (135 to 19.32 g diesel per kg soil) and pilot scale (118 to 17.5g diesel per kg soil). Table 5 shows biodegradation of diesel by using various microorganisms [67-82].

Table 5: Biodegradation of Diesel by Using Various Microorganisms.

Result of the Study	Organism Studied	References
Growth and degradation of diesel	Pseudomonas putida, Pseudomonas mallei and Enterobacter cloacae	Saadoun (2002)
Degrade diesel oil by using bioaugmentation method	*Rhodococcus sp*., Pseudomonas aeruginosa	Ueno et al. [72]
The highest degradation of diesel is 96.8%	Bacillus brevis, Pseudomonas alcaligenes, Bacillus cirroflagellosus and Citrobacterfreundii	Ahamed et al. [5]
The highest degradation of diesel is 86.94%	Acinetobactercalcoaceticus, *Acinetobacter sp.,*	
Citrobacterfreundii and Bacillus pumilus	Singh and Lin (2008)	
The highest degradation of diesel is 86.67%	*Bacillus sp* and *Bacillus Cereus*	Kebria et al. [35]
The highest degradation of diesel is 56%	Fusariummoniliforme	Dhar et al. [22]
Percentages degradation of 93% for decane, 38.4% for nonane and 22.9% for dodecane.	Rhodotorulaaurantiaca and Candida ernobii	Miranda et al. [51]
The highest degradation of diesel is 99%	Mycobacterium hyalinumand Cladosporium	Li et al. [43]
capable of utilizing diesel oil as sole substrate	Exiguobacteriumaurantiacum and Burkholderiacepacia	Mohanty and Mukherji [52]
The rates of degradation of diesel oil by the isolate at the end of day one, day twelve and day twenty-seven were 5.8 x 10-4, 1.83 x 10-3 and 1.05 x 10-3g/h,	Bacillus subtilis	Nwaogu et al. [55]
Suitable for bioremediation of diesel contamination	Ulocladiumatrum	Binsadiq [15]
The highest degradation of diesel is 55%	Acinetobactersp,	
Gordoniasp and Rhodococcussp.	Lee et al. [42]	
49.93% of diesel oil degradation in 20 days	Pseudomonas sp.	Panda SK et al. [60]

Conclusion

Petroleum hydrocarbons have devastating short-term and long term effects on soil, water as well as human health. Petroleum hydrocarbon contaminated soil affect plant growth, and reduce yield of crop from an agricultural region. Sometimes, agricultural lands become futile because of loss of fertility. Petroleum hydrocarbon contaminated water affect flora and fauna of aquatic ecosystems. Oxygen penetration is hampered and balance of marine ecosystem is ruined. As petroleum hydrocarbon is one of the main sources of fuel in the current world, the use of its products cannot be neglected. Therefore, cleanup of these worst pollutants is mandatory to keep environment safe and sound. However, petroleum hydrocarbon is not readily degradable in natural environment. Various conventional methods include physical and chemical techniques which are expensive and caused negative consequences. In such cases, bioremediation is the most effective and suitable method to remove these pollutants from the environment. A wide variety of microorganisms have the ability to degrade petroleum hydrocarbons and completely mineralize them. Phytoremediation, bioaugmentation, biostimulation etc. are some other useful bioremediation techniques to cleanup petroleum hydrocarbon from the environment.

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