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Review Article

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# Microbes and their Secondary Metabolites: Agents in Bioremediation of Hydrocarbon Contaminated Site

**Neha Sharma, Meeta Lavania*, Banwari Lal**

Microbial Biotechnology Division, The Energy and Resources Institute, New Delhi, India

***Corresponding author:** Meeta Lavania, Microbial Biotechnology Division, The Energy and Resources Institute, Lodhi Road, New Delhi, India. Tel: (+)91-11-24682100; Email: meetal@teri.res.in

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## Abstract

As the globalization increases, the demand of crude oil is also increases. The crude oil used as the fuel for the mechanical transportation of equipment or as the source of primary feedstock for many of the petrochemical industries. The petroleum hydrocarbons are major pollutant in the environment which further harms the ecosystem; the attention has been drawn to develop alternative technologies to eliminate these pollutants from the environment. Microbial remediation of hydrocarbon spills is an eco-friendly, economical approach. The microbial method of remediation is performed by addition of potential microorganisms which have tendency to utilize these hydrocarbons as a carbon source thus releasing carbon dioxide and water as by-products. Major constituents of crude oil are biodegradable so, bioremediation technique has proven to be cheap and efficient over other chemical methods. Thus, bioremediation can be considered as one of the best technologies to deal with petroleum product contaminated soil.

## Introduction

With the increasing development of the offshore oil and the transportation industry the probability of oil spill along the sides of the offshore is increasing. Petroleum hydrocarbons are the most widespread contaminants within the marine environment. Pollution by such hydrocarbons in the marine environment may be caused due to natural phenomena (natural seepages) or due to some of the anthropogenic activities such as discharge during tanks or pipelines failure [1]. The amount of natural crude oil seepage was estimated to be 600,000 metric tons per year with a range of uncertainty of 200,000 metric tons per year [2]. Release of hydrocarbons into the environment whether accidentally or due to human activities is a main cause of water and soil pollution [3].

The presence of oil in the ocean water to a great extent relies upon the mechanical (wind, waves), physical (temperature, UV) and chemical (dissolved oxygen, nutrient concentration) factors which may influence the biodegradation of crude oil. Thus, bioremediation is a process used to remediate crude oil hydrocarbon contaminants present in the soil. The major components of the crude oil are biodegradable. The process of bioremediation has been proved to be cost effective, eco-friendly and efficient than other techniques. The bioremediation of the crude oil occurs in various phases: initially, light fractions of the oil are removed by natural processes such as evaporation, photo oxidation or by geo-chemical processes; secondly heavy fractions of the oil is removed by the process of biodegradation [4]. The technology of bioremediation of these polluted sites is considered an effective biotechnology with numerous advantages as the process of bioremediation is based on the ability of the microbes to degrade the waste hydrocarbons to non-toxic products such as carbon monoxide, carbon dioxide and biomass [5]. Bioremediation can be a cost effective clean-up technology to treat the oily sludge and the degradable petroleum hydrocarbons. Bioremediation has now become a major method to be employed in the restoration of the oil polluted environments.

### Principle of bioremediation

Crude oil is a complex blend of several thousands of different hydrocarbons (aliphatics and aromatics) and nonhydrocarbons (Nitrogen, Sulphur, Oxygen (NSO) and other trace metals) [6]. The chemical composition of each category of crude oil is exclusive and different; there are diverse methods to deal with each complex component through microbial flora. Bioremediation either occurs naturally or by the addition of microbes to the petroleum hydrocarbon contaminated soil. The process through which the microbes reduce the complex organic pollutants into smaller chemical compounds is termed as biodegradation. Most of the biodegradable matters are usually organic and are generally derivatives of plant or animal matter. The micro-organisms utilize them as a carbon source and break down them by enzymatic or metabolic processes into smaller compounds to obtain chemical energy.

Biodegradation of crude oil is a complex process that mostly depends on the nature and the amounts of the hydrocarbons present in the crude oil. Bacteria are considered to be the most active agents in petroleum biodegradation. Though, the only limiting factor for biodegradation of oil in the environment is the restricted availability of the microorganisms [7]. The efficiency of microbial inoculation in soil and sediments for bioremediation and pollution control is influenced by the various factors: concentration of active inoculants, interaction between inoculated micro-organisms and indigenous population, nutrient supplies to the target micro-organisms, availability of target compound to the inoculated microorganism and effect of heterogonous materials on the biodegradation process [8].

**Microbial degradation of petroleum hydrocarbons**

All kinds of petroleum are a complex mixture of hydrocarbons and other chemicals wherein the hydrocarbons are the most abundant. The four main types of hydrocarbons are alkanes, alkenes, alkynes and aromatic hydrocarbons which all differ in their bonding characteristics [9]. The characteristics bonding between these compounds may determine a hydrocarbon's susceptibility to degradation. The susceptibility of hydrocarbons to microbial degradation can be generally ranked as follows: n-alkanes > branched alkanes > low molecular weight aromatics > cyclic alkanes. Resin and asphaltenes are the most recalcitrant compounds of the petroleum hydrocarbons [10].

Furthermore, the larger molecules are considered to be less degradable than smaller ones. Hydrocarbons in the environment are degraded primarily by bacteria, yeast and fungi. The major class of hydrocarbon degraders are bacterial community [11]. 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 [12-15]. Polycyclic aromatic hydrocarbons (naphthalene, phenanthrene, anthracene, pyrene, dibenzoanthracene, benzopyrene) constitute an important fraction of petroleum hydrocarbons and they are considered among the major contaminants in soil and water environments because many of these compounds have been found to be cytotoxic, mutagenic and potentially carcinogenic [16].

Similarly, the degradation of high molecular weight polycyclic aromatic hydrocarbons is less extensive than the high molecular weight polycyclic aromatic hydrocarbons. It has been recognised that many microorganisms have the ability to utilise hydrocarbons as the sole source of carbon and energy [17]. Microbial degradation of pollutants has been intensified in recent years (Table 1).

| Microorganisms | Applications |
|---|---|
| *Pseudomonas aeruginosa* | Bioremediation of oil contaminated soil |
| *Bacillus subtilis* | Bioremediation of hydrocarbon contaminated site |
| *Nocandiopsis incentsis* | Bioremediation of marine environment |
| *Pseudomonas cepacia* | Bioremediation of marine environment |
| *Micrococcus inteus* | Bioremediation of oil contaminated environment |
| *Pseudomonas alcaligenes* | Environmental applications |

**Table 1:** Represents the list of microbial strains involved in bioremediation.

Microbial remediation of a hydrocarbon contaminated site is accomplished with the help of a diverse group of microorganisms, particularly the indigenous bacteria present in soil. These microorganisms can degrade a wide range of target constituents present in oily sludge. Few of the petroleum hydrocarbon degraders include *Pseudomonas, Roseomonas, Yokenella sp, Bacillus sp, Flavobacter sp* etc. Other organisms such as fungi are also capable of degrading the hydrocarbons to a certain extent. However, they take a longer period of time to grow as compared to their bacterial counterparts [18].

Microbial degradation is one of the major and ultimate mechanisms for elimination of petroleum hydrocarbon pollutants from the environment. There was majorly nine bacterial strains namely *Psuedomonas fluorescens*, *Acinetobacter lwoffi, Flavobacterium sp*., *Bacillus subtilis*, *Bacillus sp*., *Alcaligenes sp., Micrococcus roseus, P.aeruginosa* and *Cornyebacterium* sp. that were isolated from the polluted stream which could easily degrade the crude oil [19]. Bacterial genera namely, *Gordonia, Brevibacterium, Aeromicrobium, Dietzia* and *Mycobacterium* isolated from the petroleum contaminated soil have the potential to degrade hydrocarbons. Similarly, fungal genera namely, *Amorphoteca, Neosartorya Talaromyces* and *Graphium* and yeast genera, namely, *Candida, Yarrowia* and *Pichia* were also isolated from petroleum contaminated soil and proved to be potential organisms for hydrocarbon degradation [20]. The yeast species, *Candida lipolytica, Rhodotorula mucilaginosa, Geotrichum* sp, and *Trichosporon mucoides* were isolated from contaminated water and have ability to degrade petroleum compounds [21].

**Bioremediation parameters**

The efficiency and the efficacy of each method of remediation have its own limitations. The goal of remediation is to eradicate the environmental pollutants. Environmental factors such as the necessities of the reaction, mobility of substances and physiological requirements of micro-organisms will affect the rate and degree of degradation of contaminants. Weathering and environmental factors may play an immense role in the success of bioremediation. Another factor that determines the rate of bioremediation process is the susceptibility of the pollutant [22].

Among physical factors, temperature plays an important role in biodegradation of hydrocarbons available in oil [23]. The simpler contaminants are easily solubilized by the microbes whereas complex or rigid molecules of the contaminants extend the process of bioremediation.

**Temperature**

Temperature tends to influence the petroleum hydrocarbon biodegradation by affecting the physical and the chemical composition of the hydrocarbon. At low temperatures, the rate of degradation generally decreases as a result of reduced enzymatic activity rates. The rate of degradation or the metabolism of the hydrocarbons reaches to the maximum extent at high temperatures ranging from 30 ºC to 40 ºC. Despite of the fact that biodegradation of the petroleum hydrocarbons can take place on a wide range of temperatures, degradation rate decreases through declining temperature [24]. At high temperature, the solubility of oxygen decrease that further limits the aerobic microbial degradation [25]. Figure1 depicts the degradation rate in soil, freshwater and marine environment.

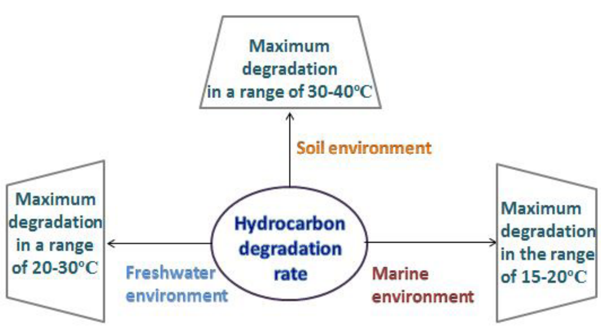


**Figure 1:** Rate of biodegradation in various environments (soil, freshwater and marine).

**Oxygen**

The concentration of oxygen has been determined as the rate limiting variable for the petroleum hydrocarbons degradation in the environment [26]. In the presence of oxygen, bacteria and fungi tend to catabolised aliphatic, cyclic and aromatic hydrocarbons through oxygenase enzyme. In several cases, the dissolved oxygen can drop to zero which leads to anaerobic degradation of hydrocarbons [27].

**Nutrients**

Inorganic sources include phosphorous, hydrogen, potassium, nitrogen or oxygen are essential for the growth and activity of the micro-organisms [28], whereas micronutrients including manganese, zinc, copper, chlorine and cobalt are required in fewer quantities. The ratios of carbon-to-phosphorus or carbon- to-nitrogen are essential factor in degradation of hydrocarbons [29].

**Concentration of hydrogen ions**

The variations in pH are less in aquatic environments and most of the bacterial and fungal degrader requires a neutral pH. The microbial metabolism is greatly influenced by low or high pH values [30]. According to Bam forth and Singleton [28], 40% of phenanthrene degradation was carried out at pH 5.5 by bacteria *Burkholderia cocovenenans.* However, the degradation of hydrocarbons at neutral pH in the same conditions was 80%. A degradation of PAH in acidic contaminated environment (pH 2.0) by indigenous microorganisms was reported [31].

**Bioavailability of hydrocarbons**

Bioavailability refers to the rate of substrate mass transfer into the microbial system. It is the most determinant parameters regarding hydrocarbon degradation rate. Polycyclic aromatic hydrocarbons are less bioavailable due to their low solubility that leads to its persistence in the environment [26]. Further, the photo-oxidation of hydrocarbons, increases their bioavailability to the microbial system [32]. Hydrocarbons and particularly polycyclic aromatic hydrocarbons become more bioavailable when they are dissolved or evaporated [33].

**Salinity**

The concentration of sodium chloride adversely affects the degradation of crude oil as it halts the activity of key enzymes present in the microbial system. Due to the presence of higher concentration of sodium chloride in the soil it tends to affect the overall microbial populations. The high NaCl concentrations are responsible for inducing osmotic shock in some bacteria, causing plasmolysis and inhibiting various physiological processes and macromolecule biosynthesis [34].

## Effect of moisture content

The moisture content of soil plays an important role in soil bioremediation. The presence of sufficient water in the soil may support the microbial activities. The limited amounts of water in the soil inhibit microbial community, while excessive water tend to fill the pores of soil and prevent the diffusion of oxygen towards the microorganisms. Thus, optimal mount of water content is essential for bioremediation [35].

## Biodegradation strategy

Bioremediation technologies for responding to marine oil spills may be divided into three discrete categories (Figure 2):

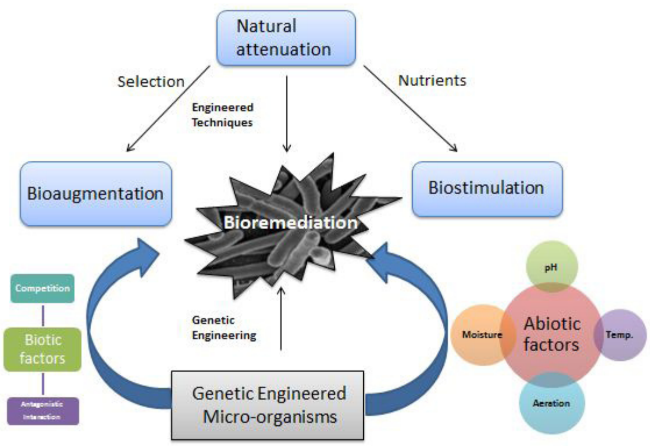


**Figure 2:** Represents biodegradation strategies of crude oil.

## Nutrient enrichment (Bio stimulation)

The lack of an adequate supply of nutrients, such as nitrogen and phosphorus may potentially affect the rate of biodegradation of hydrocarbon in the marine environment. The enrichment of nutrient has also been more thoroughly studied over other two approaches [36]. The success of the approach is depending on the oil-degrading microorganisms that were abundantly present in the marine environments and well adapted to resisting local environmental stresses. However, when oil was released in the large quantities, the limited micro-organisms are unable to degrade hydrocarbon in the absence of sufficient nutrient. The nitrogen, phosphorus and other nutrients were added to overcome these deficits and allow the degradation of hydrocarbon at optimal rate. The bio stimulation experiment in Alaska provided a wealth of experimental data about bioremediation in an open environment [37].

## Seeding with naturally occurring microorganisms (Bio augmentation)

Other approach involves the inoculation of naturally occurring microorganisms to a polluted environment that promote increased rates of biodegradation. The inoculums may be a blend of non-indigenous microbes from various polluted environments, specially selected and cultivated for their oil degrading characteristics, or it may be a mix of oil-degrading microbes selected from the site to be remediate and mass-cultured in the laboratory or in on-site bioreactors. The purpose for adding micro-organism to the spill site is that the indigenous microbial community may not include the diversity of oil degraders. The inoculated microbes not only have to degrade petroleum hydrocarbon but they would also compete with the mixed indigenous population that are well adapted to their environment. Along with this, they would have to cope with the physical conditions (temperature, water chemistry and salinity) and predation by other species [38]. The time required for introduced microbes to begin metabolizing hydrocarbons is also important. The inoculated cultures must be genetically stable, must not be pathogenic, or produce toxic metabolites. Some laboratory and small-scale experiments in controlled environments have demonstrated that seeding can promote biodegradation [39].

## Seeding with genetically engineered microorganisms (Bio augmentation with GEMs)

Though it was not evidently superior to indigenous organisms and has never been tested in the field, the frost organism ever patented was a microorganism genetically engineered to degrade oil [40]. The aim of constructing genetically engineered organisms is that they might possibly be designed either to be more efficient than naturally occurring species or to have the ability to degrade fractions of petroleum not degradable by naturally occurring species.

## Mechanism of petroleum hydrocarbon degradation

To achieve a rapid and complete degradation of the majority of the petroleum hydrocarbon pollutants was kept at aerobic conditions. Figure 3 represents the principle of aerobic degradation of hydrocarbons. The initial intracellular attack of organic pollutants was an oxidative process and the activation as well as incorporation of oxygen is the enzymatic key reaction catalysed by oxygenases and peroxidases [41]. The peripheral degradation pathways convert the organic pollutants stepwise into intermediates of the central intermediary metabolism. The biosynthesis of cell biomass occurs from the central precursor metabolites (Acetyl-CoA, succinate, and pyruvate).

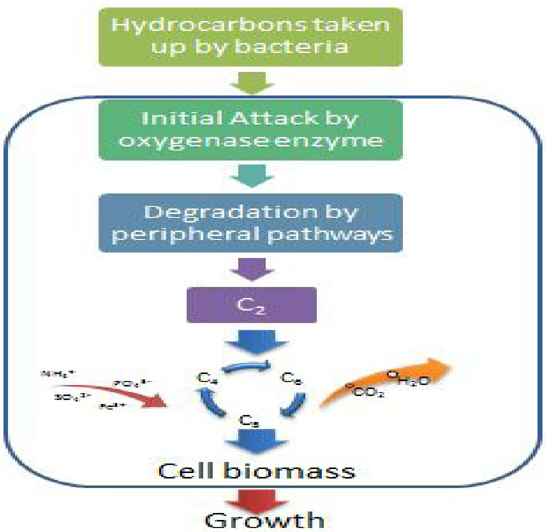


**Figure 3:** Aerobic biodegradation of hydrocarbons by microorganisms.

The degradation of hydrocarbon pollutants can also be mediated by specific enzyme system. The enzyme cytochrome P450 alkane hydroxylases constitute a super family of ubiquitous Heme-thiolate Monooxygenases which play a key role in the microbial degradation of oil, chlorinated hydrocarbons, fuel additives, and many other compounds [42]. The enzyme P450 was present only in few species that have capability to degrade petroleum hydrocarbons [43].

Other mechanisms involved are as follows: attachment of microbial cells to the substrates and Production of bio surfactants [44]. The attachment mechanism of cell to oil droplet is still unknown but production of bio surfactant has been well studied. Bio surfactant are heterogeneous group of surface active compounds produced by variety of microorganisms [45-47]. The surfactant molecules play an important role by enhancing solubilisation and removal of contaminants [48,49]. Similarly, biodegradation of petroleum hydrocarbons is also enhanced by the surfactants due to the increased bioavailability of pollutants [50].

Numerous studies have been reported that shows the effect of biosurfactant on biodegradation and their industrial and commercial potentials [51]. Various microbial species (*Acinetobacter, Stenotrophomonas, Pseudomonas, Koccuria and Bacillus*) were isolated from hydrocarbon-contaminated sites in Tunisia showed significant biosurfactant production and emulsification activities [52]. Pseudomonads are well recognized bacteria that are capable of utilizing hydrocarbons as a carbon and energy sources and producing biosurfactant [53].

Among *Pseudomonad*s, *P. aeruginosa* are extensively investigated for the production of bio surfactants. However, *P. putida* and *P. chlororaphis* are also reported to produce glycolipid type bio surfactants. Bio surfactants enhance the surface area of oil so that it can be easily accessible by the bacterial species [54]. Another report describes the potential of *Burkholderia plantarii* towards the production of rhamnolipids and their application in pharmaceutical and industries [55]. The bio-surfactants, thus, produced by the microorganisms are known to exhibit vast applications due to their specific versatile properties, non-toxic behaviour and biological acceptability [56]. Figure 4 depict the scanning micrograph of microbes that are capable of producing biosurfactant and efficiency of biosurfactant through oil spreading and drop collapse method [57].

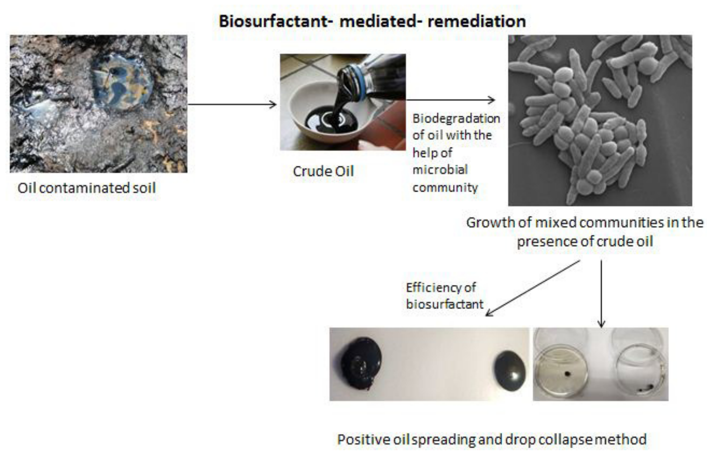


**Figure 4:** Illustrating process of bioremediation assisted by biosurfactant produced by microbial system.

## Case study of bioremediation

Bacillus cereus was capable of producing promising biosurfactant that shows 90% of emulsification activity and 60% reduction in surface tension in a mineral medium supplemented with waste frying oil under optimized conditions. The biosurfactant exhibited desirable properties for application in the bioremediation of seawater contaminated with petroleum derivatives, such as stability under various environmental conditions, dispersion capacity, and effectiveness in enhancing motor oil biodegradation in seawater samples [58]. Recent study showed that *Trichoderma reesei* H002, a filamentous ascomycete fungus isolated from a polluted site was tested for its biodegradation ability to degrade diesel oil. *T. reesei* H002 showed enhance degradation of total petroleum hydrocarbons (TPHs up to 94.78% at the end of the study (40 days) at 25 ºC [59].

*Serratia marcescens* UCP 1549 was capable of producing biosurfactant which are used in agriculture and oil spill biodegradation. The biosurfactants are surface active agents that have higher biocompatibility and biodegradability over chemical surfactants [60]. The marine bacteria isolated from Bahary area (Alexandria, Egypt), namely, *Pseudomonas* sp. sp48 displayed significant oil degradation up to 1.5%. Furthermore, it showed an ability to utilize the aromatic and aliphatic fractions of hydrocarbons [61].

TERI (The Energy and Resources Institute) has developed an Oil zapper, a consortia of oil degrading bacteria (patented) isolated from different bacterial strains present in natural environments that consume saturated hydrocarbons, aromatic component or benzene compounds, NSO (compounds of nitrogen, Sulphur and oxygen) and asphaltene and converts them into harmless $CO_2$ and water. The tendency of oil zapper was that it can work at a temperature range from 8 to 40 ºC. Implementing oil zapper for biodegradation is 30-40 % economical over other conventional treatment [62]. The report of Cappello et al. [63] and Genovese et al. [64-70], implemented a modular system for biodegradation process and tested for the remediation of hydrocarbon-contaminated sediments collected in Messina harbor (Italy). The system was developed to operate on site.

**Advantages of bioremediation**

Bioremediation has several advantages over the other remediation methods. The process of bioremediation involves the use of natural processes; it is a considered as a green method that brings about no harm to the environment. The bioremediation takes place on site which contains naturally occurring microbial species thus, it doesn't disturb the neighbouring microbial communities. The bioremediation process is cheaper and economical over other methods.

## Summary

Bioremediation of petroleum hydrocarbons in the subsurface environment is an immense problem. The mechanism of biodegradation depends on the indigenous microbial communities that transform the organic contaminants. In microbial degradation process, various physical and chemical methods were involved that critically remove the large amount of oil. Various micro-organisms utilize these hydrocarbons as a carbon and energy source. Therefore, based on the present review, it may be concluded that microbial degradation can be considered as a key component in the clean-up strategy for petroleum hydrocarbon remediation.

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## References

1. Chen Q, Li J, Liu M, Sun H (2017) Study on the biodegradation of crude oil by free and immobilized bacterial consortium in marine environment. PLOS ONE 12.
2. Kvenvolden KA, Cooper CK (2003) Natural seepage of crude oil into the marine environment. Geo-Marine Letters 23: 140-146.
3. Holliger C, Gaspard S, Glod G, Heijman C, Schumacher W, et al. (1997) Contaminated environments in the subsurface and bioremediation: organic contaminants. FEMS Microbiology Reviews 20: 517-523.
4. Bharathi B, Gayathiri E, Natarajan S, Selvadhasand S, Kalaikandhan R (2017) Biodegradation of crude oil by bacteria isolated from crude oil contaminated soil-a review. International journal of development research 7:17392-1739.
5. Netrottf MD, Albornoz E, Mostazo C, Cozzo M (1996) Bio treatment of hydrocarbons from petroleum tank bottom sludge in soil slurries. Biotechnology letter 18: 1241-1246.
6. Singh SN, Kumari B, Mishra S (2012) Microbial degradation of alkanes. In: Microbial Degradation of Xenobiotics. Berlin Heidelberg: Springer: 439-469.
7. Tsai PJ, Shied HY, Lee WJ, Lai SO (2001) Health - risk assessment for workers exposed to polycyclic aromatic hydrocarbons in a carbon black manufacturing industry. Sci total Environ 20:137.
8. Bharathi B, Gayathiri E, Natarajan S, Selvadhas S, Kalaikandhan R, (2007) Biodegradation of crude oil by bacteria isolated from crude oil contaminated soil – a review. International Journal of Development Research 07: 17392-17397.
9. Atlas RM, Bartha R (1992) Hydrocarbon biodegradation and oil spill bioremediation. Advances in Microbial ecology 12: 287-338.
10. Rojo F (2009) Degradation of alkanes by bacteria. Environmental Microbiology 11: 2477-2490.
11. Bento FM, Flavio AO, Camargo FVA, Okeke BC, Frankenberger WT (2005) Comparative bioremediation of soils contaminated with diesel oil by natural attenuation, biostimulation and bioaugmentation. Applied Bioresource Technology 96: 1049-1055.
12. Jones J, Knight M, Byron JA (1970) Effect of gross population by kerosene hydrocarbons on the microflora of a moorland soil. Nature 227: 1166.
13. Pinholt Y, Struwe S, Kjoller A (1979) Microbial changes during oil decomposition in soil. Holarctic Ecology 2: 195-200.
14. Hollaway SL, Faw GM, Sizemore RK (1980) The bacterial community composition of an active oil field in the Northwestern Gulf of Mexico. Marine Pollution Bulletin 11: 153-156.
15. Phillips GJM, Stewart JE (1974) Distribution of hydrocarbon utilizing bacteria in Northwestern Atlantic waters and coastal sediments. Canadian Journal of Microbiology 20: 955-962.
16. Celovsky H (2013) Microbial degradation of crude oil or petroleum hydrocarbons in marine environment. ENE 487 Research paper.

17. Francis M (1989) Land farming of oil wastes. Proceeding of fifth annual general meeting of BIOMINET119-127.

18. Gogai BK, Dutta A, Fedorak PM (1996) Carbazole degradation by Pseudomonas sp.LD2: Metabolic characteristics and the identification of some metabolites. Environ Sci tech 30: 575-585.

19. Das N, Chandran P (2011) Microbial Degradation of Petroleum Hydrocarbon Contaminants: An Overview. Biotechnology Research International.

20. Chaillan F, Le Fleche A, Bury E, Phantavong YH, Grimont P, et al. (2004) Identification and biodegradation potential of tropical aerobic hydrocarbon-degrading microorganisms. Research in Microbiology 155: 587-595.

21. Boguslawska-Was E, Dabrowski W (2001) The seasonal variability of yeasts and yeast-like organisms in water and bottom sediment of the Szczecin Lagoon. International Journal of Hygiene and Environmental Health 203: 451-458.

22. Harmsen J (1991) Possibilities and limitations of land farming for cleaning contaminated soils on site. Bioremediation Butterwort 255-272.

23. Andreoni V, Gianfreda L (2007) Bioremediation and monitoring of aromatic-polluted habitats. Applied Microbiology and Biotechnology 76: 87-308.

24. Pelletier E, Delille D, Delille B (2004) Crude oil bioremediation in sub-Antarctic intertidal sediments: chemistry and toxicity of oiled residues. Marine Environmental Research 57: 311-327.

25. Margesin R, Schinner F (2001) Biodegradation and bioremediation of hydrocarbons in extreme environments. Applied Microbiology and Biotechnology 56: 650-663.

26. Ghosal D, Ghosh S, Dutta TK, Ahn Y (2016) Current state of knowledge in microbial degradation of polycyclic aromatic hydrocarbons (PAHs): A review. Frontiers in Microbiology 7: 1369.

27. Boopathy R (2000) Factors limiting bioremediation technologies. Bioresource Technology 74: 63-67.

28. Bamforth SM, Singleton I (2005) Bioremediation of polycyclic aromatic hydrocarbons: Current knowledge and future directions. Journal of Chemical Technology and Biotechnology 80: 723-736.

29. Breedveld GD, Sparrevik M (2000) Nutrient-limited biodegradation of PAH in various soil strata at a creosote contaminated site. Biodegradation 11: 391-399.

30. Leahy JG, Colwell RR (1990) Microbial degradation of hydrocarbons in the environment. Microbiological Reviews 54: 305-315.

31. Shallu S, Pathak H, Jaroli DP (2014) Factors affecting the rate of biodegradation of polyaromatic hydrocarbons. International Journal of Pure and Applied Bioscience 2: 185-202.

32. Maki H, Hirayama N, Hiwatari T, Kohata K, Uchiyama H, et al. (2003) Crude oil bioremediation field experiment in the Sea of Japan. Marine Pollution Bulletin 47: 74-77.

33. Abdel-Shafy HI, Mansour MS (2016) A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. Egyptian Journal of Petroleum 25: 107-123

34. Diaz MP, Boyd KG, Grigson SJW, Burgess JG (2002) Biodegradation of crude oil across a wide range of salinities by an extremely halotolerant bacterial consortium MPD-M, immobilized onto polypropylene fibres. Biotechnol Bioeng 79: 145-153.

35. Bahmani F, Ataei SA, Mikaili MA (2018) The Effect of Moisture Content Variation on the Bioremediation of Hydrocarbon Contaminated Soils: Modeling and Experimental Investigation. J Environ Anal Chem 5: 236

36. Liu C, Zongze S (2005) *Alcanivorax dieselolei* sp. nov., a novel alkane-degrading bacterium isolated from sea water and deep-sea sediment. International Journal of Systematic and Evolutionary Microbiology 55: 1181-1186.

37. Maa FB, Jing B, Guo L, Zhao C, Chein-chi C, Di C (2009) Application of bio augmentation to improve the activated sludge system into the contact oxidation system treating petro chemical waste water. Bioresource Technology 100: 597-602.

38. Manee P, Prayad P, Edward S, Upatham A, Ladda T (1998) Biodegra-38. dation of crude oil by soil microorganisms in the tropic. Biodegradation 9: 83-90.

39. Suidan TM (1994) Effects of nitrogen source on crude oil biodegradation. Journal of Industrial Microbiology 13: 279-286.

40. Muratova AY, Turkovskaya V (2001) Degradation of petroleum oils by a selected microbialassociation. Applied Biochemistry and Microbiology 37: 155-159.

41. Fritsche W, Hofrichter M (2000) Aerobic degradation by microorganisms," in Environmental Processes- Soil Decontamination, J Klein, Ed Wiley-VCH, Weinheim, German 146-155.

42. Van Beilen JB, Funhoff EG (2007) Alkane hydroxylases involved in microbial alkane degradation. Applied Microbiology and Biotechnology 74: 13-21.

43. Zimmer T, Ohkuma M, Ohta A, Takagi M, Schunck WH (1996) The CYP52 multigene family of Candida maltosa encodes functionally diverse n-alkane-inducible cytochromes p450. Biochemical and Biophysical Research Communications 224:784-789.

44. Hommel RK (1990) Formation and phylogenetic role of biosurfactants. Journal of Applied Microbiology 89: 158-119.

45. Ilori MO, Adebusoye SA, Ojo AC (2008) Isolation and characterization of hydrocarbon-degrading and biosurfactant-producing yeast strains obtained from a polluted lagoon water. World Journal of Microbiology and Biotechnology 24: 2539-2545.

46. Kiran GS, Hema TA, Gandhimathi R, Selvin J, Thomas TA, et al. (2009) Optimization and production of a biosurfactant from the sponge-associated marine fungus Aspergillus ustus MSF3. Colloids and Surfaces B, 73: 250-256.

47. Obayori OS, Ilori MO, Adebusoye SA, Oyetibo GO, Omotayo AE, et al. (2009) Degradation of hydrocarbons and biosurfactant production by Pseudomonas sp. strain LP1. World Journal of Microbiology and Biotechnology 25: 1615-1623.

48. Brusseau ML, Miller RM, Zhang Y, Wang X, Bai GY (1995) Biosurfactant and cosolvent enhanced remediation of contaminated media. ACS Symposium Series 594: 82-94.

49. Bai G, Brusseau ML, Miller RM (1997) Biosurfactant-enhanced removal of residual hydrocarbon from soil. Journal of Contaminant Hydrology 25: 157-170.

50. Barkay T, Navon-Venezia S, Ron EZ, Rosenberg E (1999) Enhancement of solubilization and biodegradation of polyaromatic hydrocarbons by the bio emulsifier alasan. Applied and Environmental Microbiology 65: 2697-2702.

51. Batista SB, Mounteer AH, Amorim FR, Totola MR (2006) Isolation and characterization of biosurfactant/bio emulsifier-producing bacteria from petroleum contaminated sites. Bioresource Technology 97: 868-875.

52. Mahjoubi M, Jaouani A, Guesmi A, Amor SB, Jouini A, et al. (2013) Hy52. drocarbon clastic bacteria isolated from petroleum contaminated sites in Tunisia: Isolation, identification and characterization of the biotechnological potential. New Biotechnology 30: 723-733.

53. Rahman KSM, Rahman TJ, Kourkoutas Y, Petsas I, Marchant R, et al. (2003) Enhanced bioremediation of n-alkane in petroleum sludge using bacterial consortium amended with rhamnolipid and micronutrients. Bioresource Technology 90: 159-168.

54. Nikolopoulou M, Kalogerakis N (2009) Bio stimulation strategies for fresh and chronically polluted marine environments with petroleum hydrocarbons. Journal of Chemical Technology and Biotechnology 84: 802-807.

55. Hormann B, Muller MM, Syldatk C, Hausmann R (2010) Rhamnolipid production by Burkholderia plantarii DSM 9509$^{T}$. European Journal of Lipid Science and Technology 112: 674-680

56. Venkateswarulu TC, Tammineedi J (2018) Role of bio surfactants in bioremediation of oil pollution. 4: 241-249.

57. Sharma N, Lavania M, Kukreti V, Rana DP, Lal B (2018) Laboratory Investigation of Indigenous Consortia TERIJ-188 for Incremental Oil Recovery. Front Microbiol 9: 2357.

58. Durval IJB, Resende AHB, Figueiredo MA, Luna JM, Rufino RD, et al. (2018) Studies on Biosurfactants Produced using Bacillus Cereus Isolated from Seawater with Biotechnological Potential for Marine Oil-Spill Bioremediation. J Surfact Deterg 349-363.

59. Nazifa TH, Ahmad MA, Hadibarata T, Aris A (2018) Bioremediation of Diesel Oil Spill by Filamentous Fungus Trichoderma reesei H002 in Aquatic Environment. International journal of integrated engineering 10.

60. Araujo HWC, Andrade RFS, Rodriguez D, Ribeaux DR, Silva CAAD, et al. (2019) Sustainable biosurfactant produced by Serratia marcescens UCP 1549 and its suitability for agricultural and marine bioremediation applications. Microbial Cell Factories 18:2.

61. Farag S, Soliman NA, Abdel-Fattah YR (2018) Statistical optimization of crude oil bio-degradation by a local marine bacterium isolate Pseudomonas sp. sp48. Journal of Genetic Engineering and Biotechnology 16: 409-420.

62. Randhawa M, Kaushal J (2014) Bioremediation through oil zapper and oilivorous-s technologies: a review. Engineering Sciences International Research Journal 2: 29-32.

63. Cappello S, Calogero R, Santisi S, Genovese M, Denaro R, et al. (2015) Bioremediation of oil polluted marine sediments: A bio-engineering treatment. International Microbiology 18: 127-134.

64. Genovese M, Crisafi F, Denaro R, Cappello S, Russo D, et al. (2014) Effective bioremediation strategy for rapid in situ cleanup of anoxic marine sediment in mesocosm oil spill simulation. Frontiers in Microbiology 5: 162.

65. Michael J, Nimmons PE, Englert K (1992) On□site bioremediation treatment system design and on□site analytical screening of hydrocarbon□contaminated dredge sediments. Journal of Soil Contamination 1: 309-320.

66. Elektorowicz M, Yong R (1995) Feasibility Studies of Bioremediation of Hydrocarbon-Contaminated Soils, in Bioremediation of Pollutants in Soil and Water, ed. B. Schepart (West Conshohocken, PA: ASTM International 35-47.

67. Bioremediation of hydrocarbon contaminated soil. Process Biochemistry 31:514-515.

68. Das N, Chandran P (2011) Microbial Degradation of Petroleum Hydrocarbon Contaminants: An Overview. Biotechnology Research International 2011: 1-13.

69. Shukla A, Singh CS (2012) Hydrocarbon Pollution: Effects on Living Organisms, Remediation of Contaminated Environments, and Effects of Heavy Metals Co-Contamination on Bioremediation. Introduction to Enhanced Oil Recovery (EOR) Processes and Bioremediation of Oil-Contaminated Sites.

70. Nduka JK, Umeh LN, Okerulu IO, Umedum LN, Okoye HN (2012) Utilization of Different Microbes in Bioremediation of Hydrocarbon Contaminated Soils Stimulated with Inorganic and Organic Fertilizers. J Pet Environ Biotechnol 3:116.