

Oil Degradation Taking Microbial Help and Bioremediation: A Review

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Abstract

Hydrocarbons are the most efficient form hydrocarbons, due to the level of energy they produce. But the disadvantage of such effective hydrocarbons is pollution. Pollution is considered one of the most noticeable and intricate problems in the contemporary era. The widespread problem caused due to petroleum products is their discharge and accidental spillage in environment proving to be hazardous. Oil spills are a major concern these days, at industrial and developmental level. There are certain methods of clean ups like physical, chemical methods but they are really costly and skilled labor intensive. Biological method of clean-up has proved to be a good method for degradation of these PHCs. It is cost effective and eco-friendly.

Keywords: Hydrocarbons; Bioremediation; Clean up; Microbes; Petroleum; Oil spills

Introduction

The primary source of energy fuel is hydrocarbons, because of the level of energy they produce around the globe. Among all the deadly combinations of problems these hydrocarbons have on the environment or on the natural flora and fauna, pollution is at the top. Pollution is considered one of the most noticeable and intricate problems in the contemporary era. Pollution could be either organic or inorganic. The release of hydrocarbons into the environment, whether accidentally or due to human activities is the main cause of water and soil pollution. Hydrocarbon components have been known to belong to the family of carcinogens and neurotoxic organic pollutants. These hydrocarbons have been classified into different groups.

The widespread problem caused due to petroleum products is their discharge and accidental spillage in a marine environment providing to be hazardous to the surroundings as well as life forms. Oil spills are a major concern these days, at industrial and developmental level.

Hydrocarbons are considered to be of biological origin (alkanes: C10-C20; C20-C40). A lot of solid waste is generated by petroleum refineries and the oily sludge possess critical effects on the environment. The current techniques for disposal of waste like burning the sludge or burying it deep down the earth are both less effective as the burning cause the pollution again, while burying of these oily sludge's have adverse effect on the soil like mutations in the plants or destroying the good properties of soil. The disposal of these oil sludgy petroleum waste demands a lot of labor work and money.

Oil spill pollution poses a major threat to marine and terrestrial life. They lead to the loss of marine creatures. The domestic and industrial oils are dumped and left untreated on the lands, which are further been transported to water and soil and they deplete the life of underwater and terrestrial living beings, animals, fishes, and microbes, also lead to various health problems to humans. The major issue of the oil spill is that destroying the life underwater by killing marine and freshwater fishes and other living entities and on land by killing animals, by affecting humans by penetrating deep in our food chain and infiltrate through the soil to affect the plants also. When animals, fishes, and humans come in contact with the oil spilled water and soil, it hinders their digestive system, kidney disease; bone marrow damage, cause cancer decreases the level of steroid hormones and also affects in the reproduction.

This oil spilled water and soil containing high levels of toxic

compounds called polyaromatic hydrocarbons like naphthalene, Acenaphthylene, Acenaphthene, fluorene and other benzene ringed chemicals. This PAH is a threat to the environment as they are toxic, mutagenic, neurogenic and carcinogenic in nature. Thus, there is an utmost need of degrading these polyaromatic hydrocarbons to reduce the burden on the environment. As these PAHs enter in water streams, soil, taken up by plants and enter the food chain, they could not be recycled and destroyed by any means.

Thus, remediation of these hydrocarbons by natural decontamination process is of utmost importance. Biodegradation is required thus to convert these complex hydrocarbons into simple that are less toxic and least problematic to the environment. Bioremediation is a non-invasive and cost-effective technique for the clean-up of these petroleum hydrocarbons. In this study, we have investigated the ability of microorganisms present in the sediment sample to degrade these hydrocarbons, crude oil in particular so that contaminated soils and water can be treated using microbes.

Bioremediation is a promising option for the complete removal and destruction of contaminants. Bioremediation is the use of living organisms, primarily microorganisms, to degrade or detoxify hazardous wastes into harmless substances such as carbon dioxide, water, and cell biomass. As such, it uses relatively low-cost, low-technology techniques, which generally have a high public acceptance. PAHs are biodegradable and bioremediation for clean-up of PAH wastes has been extensively studied.

Physical Clean-up methods like mechanical, burying, evaporation, dispersion, washing etc. and chemical methods like volatilization, photo-oxidation, chemical oxidation, and bioaccumulation etc. of these hydrocarbons and petroleum derivatives of waste and spilled oils are really expensive. These methods are not safe not cost-effective, not even quarantine, lead to incomplete decomposition of contaminants, not rapid, successful and can't remove trace amount of these hazardous pollutants [1]. Biological methods are cheap and better methods to degrade the oil spills and petroleum containing soil [2,3]. Successful

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Received December 20, 2018; Accepted March 15, 2019; Published March 22, 2019

Citation: Rathi V, Yadav V (2019) Oil Degradation Taking Microbial Help and Bioremediation: A Review. J Bioremediat Biodegrad 10: 460.

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degradation of much solid oil contaminated sludge's, soil and even water samples are reported from last two decades. These degradations are done using the microbes as the primary source of degrading these oil contaminated samples. There are numerous examples of bioremediation in research area which have opened new doors to this bioremediation technique. Successful degradation of benzene in the aerobic and anaerobic environment has been reported. The degradation was comparatively slow and poor under the anaerobic conditions [4].

Petroleum and its hydrocarbons

Petroleum also called crude oil is found in the deep bed of mother earth and is giving many other forms of hydrocarbons other than petrol. It is basically formed when the microbes and plants go on buried, their fossils got converted to other forms for over millions of years and thus called fossil fuel. They have C-H functional group. With the fractional distillation of this crude oil, we get different types of hydrocarbons that are classified according to the source and age of the fossil fuel and that are used by mankind for their own good in different forms.

Complex mixtures of paraffin (15-60%), naphthalene (30-60%) and aromatics (3-30%) are making up the crude oil. These are basically the aliphatic and aromatic chains of alkenes, cycloalkanes and polyaromatic hydrocarbons (PAHs). Crude oil is basically undergoing fractional distillation to make products like petrol, diesel, and kerosene oil.

An eye on pollution petroleum hydrocarbons

Organic pollution is more troublesome hydrocarbon form, which is petroleum, which includes aliphatic, aromatic compounds alongside alkanes and other minor constituents [5,6]. Crude oil is the origin of petrol and other petroleum products whose major constituents are hydrocarbons [7]. The main aromatic hydrocarbon in petroleum products is Benzene, toluene, and xylene [8]. These hydrocarbons have said to have a biological origin [9,10]. Aromatic compounds are benzenes that are either mono or polycyclic called as PAH which are two fused benzene rings arranged in different configurations like linearly, angularly or in cluster form [11].

This PAH is a threat to the environment as they are toxic, mutagenic, neurogenic and carcinogenic in nature [12,13]. Also, they are difficult to be removed from the environment due to their property of being hydrophobic in nature which increases with increase in molecular weight and leads to higher toxicity and extended retain met in the environment.

Origin of Oil Spills

Hydrocarbons enter into the environment through waste disposal, accidental spills, as pesticides and via losses during transport, storage, and use and their accumulation in the environment causes serious problems. Oil spills that occur during discharge from the refineries, accidents of ships/tankers, their grounding, rupture on the seabed and onshore pipelines, offshore oil production, and exploration platforms do affect these habitats causing irreversible damage to the biodiversity. Refinery effluent and oil spills are the major sources of oil pollution. According to data from last 2 decades, the natural crude oil seepage is reported to be 600,000 metric tons a year with a standard deviation of 200,000 metric tons per year [14]. These hydrocarbons invade the environment through series of events like waste disposal, accidental spills by ships and submarines, as herbicides and pesticides, lead to contaminated land, air and water streams [15]. Over a decade many clean-up methods have been developed and applied [16].

Sources of PHC Pollution

Accidental oil spillages are the main sources of these petroleum hydrocarbons (PHCs). These spillages occur either routinely or sometimes during the transportation of petroleum or crude oil extracted products like diesel or kerosene. Other causes could be the seepage from the natural deposits, pipeline tanks leakage and seepage leading to contaminated soil and water bodies (Tables 1-3). These oils spilled either accidentally or naturally causes the crude oil to mix with the soil or water bodies causing great harm to plants and marine species of plants and animals.

Indian Scenario

India is the 7th largest country with second largest in terms of population with great consumption of energy and resources. Over 35% of energy resources are consumed in India per year making it more than 40 million metric tons per year. It is estimated that by 2020-2021 the consumption of these energy resources will be 240 million metric tons and will increase by 51% by 2030.

Different methods of clean up the oil spilled soil and water

Different methods are there to clean up the soil and water contaminated due to petroleum hydrocarbons like physical, chemical and biological.

- **Physical clean-up methods** like mechanical, burying, evaporation, dispersion, washing etc. That implies with cleaning and

| Product | Lower Carbon Limit | Upper Carbon Limit | Lower Boiling Point (°C) | Upper Boiling Point (°C) | Lower Boiling Point (°F) | Upper Boiling Point (°F) |
|-------------------------|------------------------------|--------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Refinery gas | C ₁ | C ₄ | -161 | -1 | -259 | 31 |
| Liquefied petroleum gas | C ₃ | C ₄ | -42 | -1 | -44 | 31 |
| Naphtha | C ₅ | C ₁₇ | 36 | 302 | 97 | 575 |
| Gasoline | C ₄ | C ₁₂ | -1 | 216 | 31 | 421 |
| Kerosene diesel fuel | C ₈ | C ₁₈ | 126 | 258 | 302 | 575 |
| Aviation turbine fuel | C ₈ | C ₁₆ | 126 | 287 | 302 | 548 |
| Fuel oil | C ₁₂ | >C ₂₀ | 216 | 421 | >343 | >649 |
| Lubricating oil | >C ₂₀ | | >343 | | >649 | |
| Wax | C ₁₇ | >C ₂₀ | 302 | >343 | 575 | >649 |
| Asphalt | >C ₂₀ | | >343 | | >649 | |
| Coke | C ₈₀ ^a | | >1000 ^a | | >1832 ^a | |

^aCarbon number and boiling point difficult to assess; inserted for illustrative purposes only.

Table 1: Classification of petroleum.

ultimately purifying the soil and water that are polluted due to petroleum hydrocarbons.

- **Chemical methods** like volatilization, photo-oxidation, chemical oxidation, and bioaccumulation etc. of these hydrocarbons and petroleum derivatives of waste and spilled oils are really expensive. These methods are not safe not cost-effective, not even quarantine, lead to incomplete decomposition of contaminants, not rapid, not successful and can't remove trace amount of these hazardous pollutants [1,13,17].

Bacterial Degradation

Bacterial degradation of petroleum has been reported and known for around last 5 decades and the bacteria have been isolated from oil spills in soil and water [18]. In recent years many ecologist and biologists have found many bacteria that can degrade these hydrocarbons efficiently. Many oil-degrading bacteria have been isolated and their degradation potential is investigated. Major Bioremediation studies have been conducted using pure cultures while these bacteria's role remain silent in a natural environment [19].

Numerous reports have been there on the bacteria's as a proof and evidence that has the ability to degrade the oil spill water and soil that

is contaminated due to petroleum hydrocarbons, which has happened to be there due to oil spills only [20].

Biological method of clean up

Biological methods are cheaper and better methods to degrade the oil spills and petroleum containing soil [2,3]. Biological methods include the use of microbes or by using the enzymes of the microbes to detoxify and remove the pollutants from soil and water have diverse metabolic capabilities [21,22].

Bioremediation

Bioremediation is not an out of the blue type technology. It is known that bacteria has got the potential to degrade almost every type of compound. The time of degradation varies basically. As plastic takes around 400 years to degrade, some compounds take much lesser type depending on the physiological condition and type of bacterial strain degrading the compound. Biodegradation of hydrocarbons is possible through many strains of bacteria [23].

Environment contamination via these hydrocarbons was reported around 3 decades ago and still, there are numerous reports on how these hydrocarbons are aiding in environmental pollution, degrading the quality of soil, affecting microbes that are living in the deep earth

| Constituents | Problems with soil | References | Problems with Microbes | References |
|---------------------|--|--|---|------------|
| Crude oil n alkanes | Form continuous cover on soil, decaying and mixing up with hummus, making it enriched with humic acids. The degree of humification of organic matter of soil gradually decreases | [43] | Crude oil along with n alkanes are toxic to microbes α-pinene, limonene, camphene, and isobornyl acetate are proved to be toxic | [44] |
| | Lower water holding capacity, lower hydraulic conductivity and lower moisture content | [45] | The bacterium is having increased lag phase and decreased growth rate | [46] |
| | Accumulation of aluminum and manganese ions that hinders the plant growth creates an anaerobic condition in soil and waterlogging | [43] | Toxic effects of cyclohexane including inhibition of oxygen uptake and disrupted membrane potential was seen in yeast <i>Saccharomyces cerevisiae</i> . | [47] |
| | Decrease agricultural productivity of the soil | [48] | Accumulation of cyclic hydrocarbons in microbial cell membrane has effects on structural and functional properties | [49] |
| | | Alter of membrane structure by changing the fluidity and proteins structures or patterns and ultimately disruption of energy flow and transduction | [50] | |

Table 2: Effects of PHCs.

| Constituents | Harmful effects on plants | References | Harmful effects on animals, birds, fishes, and animals along with human body | References |
|---|--|------------|---|------------|
| Crude oil and PHCs | PHCs prevent the entry of oxygen and water to seed by forming a composite film | [51] | If ingested accidentally may cause serious health problems to animals and birds like pneumonia, emphysema, congestion or even death due to choking by fumes of hydrocarbons or PHCs | [52] |
| Diesel | Lower germination rate (16-20%) in species like <i>Bromous Mermis</i> , <i>Secal seral</i> , <i>Triticum Sativa</i> and <i>Agropyron deserterum</i> , <i>Arachis hypogaea</i> , <i>Vigna unguiculata</i> , <i>Sorghum bicolor</i> , <i>Triticum aestivum L.</i> and <i>Zea mays</i> . The effect also is seen in shoot height, root length, plant height | [53-55] | Peroxidation in liver tissue and kidneys of fish Decreased absorption of nutrients in animals and birds | [56-57] |
| | The decrease in chlorophyll content, decrease in amylase content, starch phosphorylase and increase in sugar content | [58] | Xylene vapors Caused the subsequent decrease in total white and red blood cells count, decreased enzyme alkaline phosphatize, bilirubin inconsistency in dogs and rats | [59] |
| Gasoline fuel/diesel | Decreased seed germination, dry weight, leaf area, root and shoot height, reduction in radicle and plumule growth | [60] | mutagenic and possess many immuno-toxicants that are a threat to human and animal health | [61-65] |
| Effect of PHCs on Human | | | | |
| Aromatic hydrocarbons like benzene, toluene, and xylene (BTX) | Prolonged exposure to BTX cause lung, liver, kidney disease and bone marrow damage and cause cancer | | | [48,66] |
| | Long-term mental health issues, lower respiration, genotoxic damage, hormonal problems, reproductive damage, skin and lung cancer | | | [67] |
| | mutagenic and possess many immuno- toxicants that are a threat to human | | | [64,65] |

Table 3: Effects of PHCs on Plants, Animals, and Humans.

bed and affecting the lives of animals and plants. With the help of microbes, the waste material could be converted to organic material like carbon dioxide, methane, and water [24].

Contamination of environment with these hydrocarbon has caused critical health defects and due to this, there is increased consideration been centred on creating and executing imaginative innovative technology and techniques for cleaning the environment [25]. Bioremediation strategies over the years have proven to be useful and have effectively got great attention as a promising mother earth-friendly method for the remediation of hydrocarbon by utilizing these microbes [26]. This approach could be set because of the microbial availability and the ability of microbes to degrade such types of dangerous PHCs. These microbes utilize them as their energy source and degrade them using their enzymes [27].

Biological method using microbes: An efficient approach

Bacterial isolates produce their source of carbon and energy by degrading the hydrocarbons [28]. Biodegradation by the microbial load is the basic mechanisms through which all the diverse petroleum hydrocarbons along with minor constituents could be degraded and removed successfully by converting the complex and hazardous substances to into simpler and nontoxic forms [3,29,30]. Microbes should be provided with adequate amount of nutrients, oxygen, and pH between 6 to 9 so that they can exploit all their capabilities to degrade hydrocarbons efficiently [13]. Bacterial cell wall has some oil containing molecules called hopanoids indicating the biodegradation of oil products [9,31]. Use of surfactants has been recorded to enhance degradation of crude oil [32,33]. Microbes degrade the petroleum hydrocarbons like benzene aerobically much faster than anaerobically as the rate of degradation is slow and poor in an anaerobic environment [34]. There are around 22 species of bacteria's that degrade the petroleum hydrocarbon naturally like *Pseudomonas*, *Aeromonas*, *Bacillus*, *Flavobacterium*, *Corneybacterium*, *Micrococcus* etc. Based on the oil-degrading capacity *Pseudomonas* species is the most efficient petroleum degrader [35,36].

There are 4 approaches to biological oil spill bioremediation

- 1. Bioaugmentation:** Oil-degrading bacteria are added as a supplement to help the already existing microbial population to degrade the hydrocarbon. Addition of specific microorganisms, native or exogenous to the contaminated sites for effective bioremediation. Bioaugmentation can be carried out by inoculating whole cells or encapsulating the cells in a carrier material.
- 2. Biostimulation:** Addition of nutrients and growth limiting co-substrates to support the growth of indigenous oil degraders. Here there is the utilization of an indigenous variety of microbes. No additional supplementation of nutrients required.
- 3. Bioventing** uses microbes to degrade those organic constituents that are present on soil or either adsorbed on the soil. This bacterial activity is even increased by providing aeration condition to bacteria. So aerobic degradation is more successful in the case of venting. Lee and Swindoll showed the accuracy of bioventing for the *in-situ* degradation of light hydrocarbons like petrol and diesel and other forms of aromatic compounds.
- 4. Biosparging** is similar to the bioventing concept. It involves the supply of air under pressure in a zone that will be saturated so as to vaporize the volatile contaminants. These vaporized

contaminants get carried away to the unsaturated zone where the further work is done by microbes as microbes degrade these volatile contaminants in that unsaturated zone. It is used for the degradation of aromatic and cyclic hydrocarbons. Compounds like gasoline, diesel, and kerosene could be degraded using this biosparging technique. Natural microbes like *Candida*, *Magneto bacterium*, *Flavobacteriales bacterium*, has the potential to break these volatile harmful compounds.

Current oil removal technologies available

Remediation by enhanced natural attenuation (RENA) is a land farming treatment technology for intervention in petroleum hydrocarbon contaminated soils [37-40]. RENA is a full-scale bioremediation technology in which contaminated soils, sediments and sludges are periodically turned over or tilled into the soil to aerate the waste. Soil conditions are often controlled to increase the rate of contaminant degradation [41,42]. The RENA technique is a very effective way of carrying out bioremediation, which helps soils, contaminated with crude oil reach the ALARP condition. The microbes make use nitrate and phosphate in the degradation process. Looking forward to all the oil spill degradation techniques, Biological remediation using microbes i.e., Bioremediation holds the promise of degrading the hydrocarbons present in the oil contaminated soil and water.

Future scope

Major PHCs are contaminating the environment and are responsible for environmental pollution. They are affecting the soil and water to a great extent. They possess harmful effects that are life taking too. These hydrocarbons create the pollution that is fatal to some species of mother earth. They have a direct effect on soil, microbes, plants, animals, and humans too. As discussed in the review, it is also seen that degradation of these harmful PHCs is also possible via many different ways like physical, chemical and biological methods. They are called the clean-up methods usually. They have good results and great potential to degrade these poly hydrocarbons. The cost for the physical cleanups like mechanical, burying, evaporation, dispersion, washing, and chemical clean-up methods like volatilization, photo-oxidation, chemical oxidation, and bioaccumulation are really costly. Their cost is enormous. The cost includes machine setups, labour outputs, skilled person salary etc. The government of any country does not invest such big amounts on the degradation of these hydrocarbons. Due to lack of public acceptance and complexities of technology, these methods could not be applied successfully. So, biological degradation methods are generally applied as they are easy to achieve. These methods include bioaugmentation, biostimulation, bioventing, and sparging. These methods are not only cost-effective but also eco-friendly as all the work is done by the microbes only. There is only the need to find the robust microbe, efficient enough to degrade this lethal hydrocarbon pollution. In future, this biological degradation of soil contaminated with oil will become trendy as it is cost effective and involves the use of microbes. Microbes will have the carbon and nitrogen source in the form of pollutants like diesel and gasoline. Microbes only need optimum conditions like temperature and pH and supplements to utilize the carbon source as energy and degrade those hydrocarbons converting the inorganic waste to organic one in the form of carbon dioxide, methane and water.

Conclusion

Due to oil spill lot of pollution is affecting the environment like aquatic or marine species, plants, animals, humans, soil, and micro-organisms. They are badly affected by the oil spills for example in the

1967 "The Torrey Canyon oil spill" one of the biggest and first oil spills it was around 25 to 36 gallons of oil was spilled in the Scilly Isles, U.K that affect 180 miles of coastland area and numerous aquatic and marine species. Likewise, there are many accidental spills like the sea star oil spill in Oman, Odyssey oil spill in Canada and Gulf oil spill. The later one is world's largest accidental spill reported in the world history, in which approximately 206 million gallons were spilled into the sea (MNN, 2010). So, these type of oil spills are not easy to clean by the physical and chemical method, these methods are not cost-effective and result in depletion of aquatic and marine life forms. Biological methods where microbes are used for degradation of oil spills. By biological methods like bioremediation and phytoremediation, we can clean it very easily, cheaply and rapidly with the help of microbes. Specific microbes isolates will help to degrade oil very easily. The biological method of clean-up is cost effective and environmental friendly. The present focus of all the researchers is on the isolation and characterization of an effective hydrocarbon (especially PAH) degrading microbes from oil-contaminated environment. This technique of bacterial isolation (bioremediation) from a contaminated site, isolating it, identifying and then make use of this strain to degrade the pollutants is opening new doors for the bioremediation industry and there lies a good future of mother earth in hands of microbes.

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