Bioremediations for Oil Spills by Utilizing Microbes

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1 Introduction

An oil spill is a seepage from ocean-going tankers, pipelines, or any other oil sources. It happens mostly and becomes a reason for immense ecological harm. Bioremediation for oil spills is a technique during which microorganisms are used to eliminate the contamination of hydrocarbons from soil and water and so make them sheltered for terrestrial and aquatic species. Bioremediation can be done by using bacterial species, fungal species, and plant species. Bioremediation that is made by utilizing fungal species is called mycoremediation, and the remediation that is made by the plant species is called phytoremediation. The spilled oil in the oceans destroys the earth's ecosystem and also have an extremely negative impact on the existing creature. The contaminant materials present in oil affect the entity of marine life. We know about the oil rigs and also how the oil is detracted from the sea bedding.

This extracted oil is utilized for multiple purposes, such as carriage, construction, and processes in various industries. During loading or unloading, ballasting, and tank cleaning, the oil spilled by tankers causes ocean contamination. An oil lapse is the leakage of fluid petroleum hydrocarbons into the climate by the action of humans. Contamination of marine water depends on which type of oil is suddenly dropped into the marine water. Any type of crude oil that spills into the ocean can be any type of crude oil or pure petroleum products such as oil mixed in waste, or oily refuse, gasoline, or diesel fuel. Whether light oil such as diesel oil is spilled, this oil does not remain in the atmosphere for a prolonged period of time because it evaporates readily, although it is toxic and highly flammable (Liu et al., 2010).

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R. A. Bhat et al. (eds.), *Microbial Bioremediation*,
https://doi.org/10.1007/978-3-031-18017-0_4

2 Petroleum

Petroleum is a much more complicated combination of a large variety of high- and low-molecular-weight hydrocarbons. This complicated combination of petroleum contains branched alkanes, saturated alkanes, naphthenes, alkenes, and aromatics, including aromatics containing hetero atoms like oxygen, sulfur, nitrogen, and different heavy metal complexes, large aromatic molecules like resins, asphaltenes, naphthene aromatics, and the hydrocarbon containing various functional groups like ethers and carboxylic acids. Heavy metals are present in unrefined oil, which is linked to pyrrolic structures known as porphyry (Tang et al., 2019). Petroleum is exposed to living matter in numerous ways, indirectly or directly.

There are certain products that are built up during petroleum rectification and processing that are utilized for the generation of different products that are supreme toxicants. Incessantly, these toxicant compounds are unintentionally liberated into the atmosphere and so on; hydrocarbons constitute the prime cause of atmospheric contamination. The toxicity of hydrocarbon particles and their utility to microbial metabolism depends upon their physical and chemical nature. Chemicals present in unrefined oil causes a variety of hazardous health impacts on human and animals, depending upon the degree of exposure and sensibility. The toxicant chemicals present in unrefined oil are able to harm organs of the human body such as the immune, nervous, respiratory, reproductive, circulatory, endocrine, and sensory systems and hence cause a broad spectrum of illness and deformation (Liu et al., 2010; Tang et al., 2019). The deformation due to the toxicity of unrefined oil to body systems can be instant, or this can take up numerous months. In spite of that, oil refineries produce a large amount of oily slush, which is a hydrocarbon waste. The Exxon Company and the US Environmental Protection Agency utilize microbes to clean Alaskan beaches that get contaminated by the Valdez oil spill, the process called bioremediation (Tang et al., 2019). There are physical, biological, and chemical methods. Physical modes comprise truck vacuums, skimmers, and booms. Chemical modes comprise surface collecting agents, dispersants, and surface washing agents. In the biological mode, microbial cultures, nutrient additives, and enzyme additives are used to enhance the rate of degradation of the pollutant (Tang et al., 2019; Atlas, 1981). In India, bacterial species have evolved to change oily slime and oil spills. Oil zapper or inoculant is impressive in spacious field trials as well (Singh & Singh, 2007). Bacteria and fungi chiefly biodegrade the hydrocarbons in the atmosphere. For soil fungi, the range of biodegradation is 6–82%, and for soil bacteria, it is 0.13–50% and 0.003–100% range for marine bacteria. Several scientists reported that mixed populations with enzymatic efficiency are expected to degrade complex combinations of hydrocarbons like unrefined oil in soil, marine environments, and fresh water. In petroleum degradation, bacteria are the most effective agents, and they chiefly work to degrade the spilled oil in the environment (Atlas, 1981). Bioremediation is done with the help of bacterial species, plant species, and fungal species (Wang et al., 2008; Bahadure et al., 2013).

3 Components That Influence Petroleum Hydrocarbon Degradation

There are a different number of factors that influence the biodegradation of petroleum hydrocarbons. Many of them are discussed here (Brusseau, 1998).

1. The conformation and the implicit biodegradation capability of the petroleum hydrocarbon contaminants is the first and predominant important consideration when the eligibility of a remediation approach is to be judged.
2. Among all the physical factors, temperature plays a significant role in the degradation of petroleum hydrocarbons by directly affecting the pollutants as well as by affecting the physiology and diversification of the microbial flora. At low temperatures, the viscosity of the oil is enhanced, while the volatility of low-molecular-weight hydrocarbons is reduced, so the degeneration rates decrease (Atlas, 1975). The solubility of hydrocarbons is also affected by temperature (Foght et al., 1996). Even the biodegradation of hydrocarbons can occur over a broad range of temperatures; the rate of biodegradation of petroleum commonly decreases with decreasing temperature. The temperature range at which the degradation is highest is in the range between 30 and 40 °C in the soil, in fresh water it is 20 and 30 °C, and in the marine environment it is 15 and 20 °C (Bartha & Bossert, 1984). Venosa and Zhu (2003) have stated that the vast temperature of the atmosphere impresses the characteristics of lapsed oil and also the activities of microbes (Pelletier et al., 2004; Delille et al., 2004).
3. Nutrients are also significant ingredients in the prosperous degeneration of hydrocarbon pollutants, particularly phosphorus, nitrogen, and iron (Cooney, 1984). Atlas (1985) reports that when a large oil spill occurs in freshwater and marine environments, supplies of carbon are enhanced and the availability of nitrogen and phosphorus commonly becomes the limiting factor for oil degeneration. In oceanic environments, it gets more clear due to low levels of nitrogen and phosphorous in seawater (Floodgate, 1984). Freshwater wetlands are typically nutrient deficient because of the bulky demands for nutrients by the plants. So, the summation of nutrients is necessary to increase the biodegradation rate of oil pollutants (Choi et al., 2002; Kim et al., 2005). In spite of that, intense nutrient concentrations may also prevent the degradation rate (Chaillan et al., 2006).
4. The negative effects of high NPK levels on the biodegradation of hydrocarbons have also been reported (Oudot et al., 1998; Chaineau et al., 2005), specifically on aromatics (Carmichael & Pfaender, 1997). The impact of fertilizers on unrefined oil bioremediation has also been studied (Pelletier et al., 2004).
5. Utilization of poultry compost as a biofertilizer in polluted soil was also studied (Okolo et al., 2005), and the biodegradation rate also increased in the presence of poultry manure. Photo-oxidation also enhanced the degradation rate of petroleum hydrocarbons by enhancing their bioavailability (Maki et al., 2001).

4 Mode of Action of Petroleum Hydrocarbon Degradation

Under aerobic conditions, very fast and complete degeneration of biotic pollutants occurs. Activation as well as inclusiveness of oxygen is the enzymatic reaction catalyzed by the enzymes oxygenases and peroxidases. The preparatory intracellular invasion of biotic contaminants is an oxidative process (Fig. 1).

The peripheral degradation pathways modify biotic pollutants into intermediates of the central intermediary metabolism, for instance, the tricarboxylic acid cycle. Biosynthesis of cell biomass occurs from the central precursor metabolites, for example, succinate, acetyl-CoA, and pyruvate. The degradation of petroleum hydrocarbons can be mediated by a specific enzyme system.

5 Bioremediation

The word bioremediation consists of two parts: bio and remediation. "Bios" means live organisms and the word "remediate" means to resolve an issue. So, the term "bioremediation" means utilizing biotic organisms to resolve an environmental issue such as contaminated soil, oil spills, or polluted groundwater. Bioremediation is the application of live microbes to degrade atmospheric contamination. Bioremediation is a technique to recapture environmental pollutants and so restore pure natural surroundings and prevent the environment from further contamination (Sasikumar & Papinazath, 2003). Bioremediation can easily be defined as a biotic process of cleaning the contaminated atmosphere. The environment can be terrestrial, aquatic, or both (Sardrood et al., 2013). In bioremediation, microbes have been applied for the treatment and alteration of waste products. Bioremediation is considered a new technology for the eco-friendly decontamination of contaminated atmospheres (King et al., 1997). Bioremediation is a naturalistic process and so it is understood by most people as an admissible waste treatment process for depraved

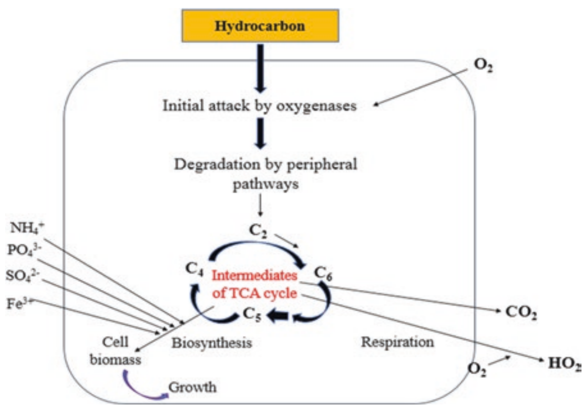

Fig. 1 Mechanism of degradation of petroleum hydrocarbon

materials such as soil and the ocean. Microbes have the potential to degrade pollutants. They increase in numbers when the contaminant is present, and when the pollution is degraded, the biodegradative microbe population falls. The remaining residues from the treatment are commonly harmless products and comprise carbon dioxide, water, and cell biomass. In nature, there are sufficient bioremediants that are used against a wide range of pollutants, and bioremediation is considered a practical technique for the overall degradation of a broad range of contaminants. Numerous compounds that are legally considered injurious and dangerous can be changed into harmless products. Bioremediation saves the bioweb and prevents the passage of hazardous and dangerous contamination from one ecosystem to another. Most of the bioremediation can be carried out onsite, often without causing a general disintegration of normal activities. This also displaces the need to transport the waste to the site and the potential risk to human health and the environment, which can rise during transportation. Bioremediation has proved less costly than other technologies that are used to clean up risky waste (Vidali, 2001). Yet, bioremediation technology bears two drawbacks. One drawback is that only certain bacteria and fungi work on a wide range of organic compounds. So there are not enough microbes to destroy chemical contamination in nature. Another drawback to bioremediation is that it takes a prolonged time to act and inflict its effect. Certain solutions are there to make us free of such a limitation. By using genetic manipulation techniques, an invaluable opportunity has been obtained to enhance new strains of bioremediation. Bioremediation works only on those compounds that are biodegradable. Biological processes are frequently highly specific. Significant factors required for well-turned bioremediation comprise the existence of metabolically able microbial populations, appropriate levels of nutrients and contaminants, and suitable environmental growth conditions. Contaminants can exist in the form of solids, liquids, and gases. Frequently, bioremediation takes longer than other treatment options, like excavation and incineration (Vidali, 2001). Although bioremediation is considered a credible technique for present environmental problems, it can also be considered problematic since additives used to encourage the activity of special microbes may disrupt the habitats of other microbes living in the same environment. Furthermore, genetically modified microorganisms that are liberated into the environment for a fixed period of time become difficult to remove. Bioremediation is very costly, and it takes several months of labor to complete the remediation of polluted environments. Nutritional imbalance can prevent biodegradation. An inadequate diet of nitrogen, phosphorus, potassium, and sulfur can limit the rate of degradation of hydrocarbons in the environment (McGill & Nyborg, 1975). There are adequate hydrocarbon-utilizing microorganisms in the soil that help in bioremediation as soon as nutrient limitation is reduced (Stone et al., 1942). Soybean lecithin, natural phospholipids, and ethyl allophanate are the best available nitrogen and phosphorus sources for the microbial bioremediants of oil contamination (Olivieri et al., 1978). No doubt, bioremediation is a necessity in the current world and can lead to the maintenance and preservation of natural resources.

6 Types of Bioremediation

Bioremediation is of two types on the basis of the place where wastes are removed, i.e., in situ bioremediation and ex situ bioremediation.

1. *In Situ Bioremediation*: In situ bioremediation is applied to eliminate the pollutants in contaminated soils and from groundwater. It is a preferable method to clean up contaminated environments since it saves transportation costs and, in it, harmless microorganisms are used to eliminate chemical contamination. These microbes have a better positive chemotactic affinity toward contaminants or pollutants. The next advantage of in situ bioremediation is the workability of isochronous treatment of soil and groundwater. Yet, in situ bioremediation also has some disadvantages, such as the fact that this method is more time-consuming than other remediation methods (Fig. 2).

   There are two types of in situ bioremediation that are distinguished based on the origin of the microorganisms applied as bioremediants.

   (1) *Intrinsic bioremediation*: It is carried out without direct microbial amendment and via intermediation in the ecological conditions of the contaminated region and the metabolic activities of naturally existing microfauna by improving nutritional and ventilation conditions.
   (2) *Engineered in situ bioremediation*: In this kind of in situ bioremediation, certain microbes are introduced to a contaminated site.

2. *Ex Situ Bioremediation*: Ex situ bioremediation is a process of bioremediation that takes place anywhere away from the contamination site, and so it needs transportation of contaminated soil or pumping of groundwater to the site of

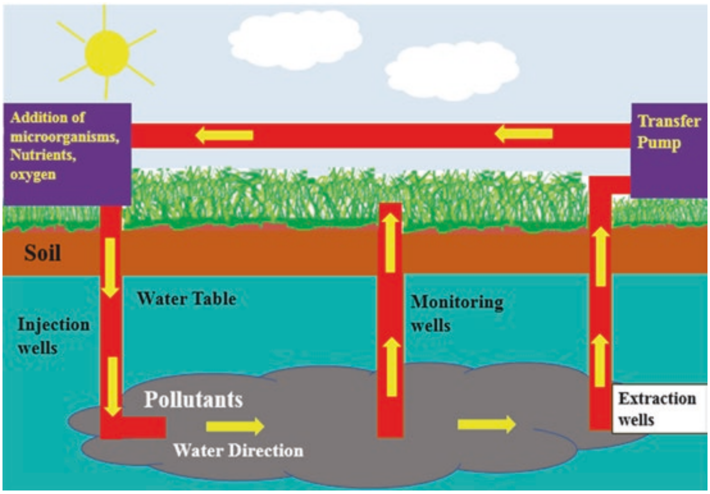

Fig. 2 In situ bioremediation to decontaminate groundwater and soil

bioremediation. This ex situ bioremediation technique has more disadvantages than advantages, as the steps of ex situ bioremediation are classified as follows:

(1) *Solid phase system*: Solid phase treatment includes treatments of land and soil. The system is used to cure domestic and industrial wastes, organic wastes, sewage sludge, and municipal solid wastes. In solid-phase soil bioremediation, these processes include land-farming and composting.
(2) *Slurry phase systems*: Solid–liquid suspensions in bioreactors are an example of slurry phase systems. Slurry phase bioremediation is a comparatively faster process than the other treatment processes. Contaminated soil is assorted with water and additives in a big tank called a bioreactor and combined to bring the indigenous microbes in nearby contact with soil contaminants. The optimum conditions in the bioreactor are adjusted so that an optimal environment for microbial bioremediation is provided. After completing the process in the bioreactor, the water is removed, and the remaining waste solids are disposed of.

7 Different Bioremediation Methods to Cure Oil Spills

Various techniques are employed either in situ or ex situ to eliminate toxic substances from the soil. The utility of techniques depends on the form and the intensification of the pollution. With the help of enzymes that are secreted by microbes, breakdown of the toxic compounds takes place. So, the water or soil becomes clear when the chemicals are taken up by the microbes (Wang et al., 2008). Bioremediation for oil spills is a technique that removes the contamination of hydrocarbons from water and soil. Oil leakage occurs mostly from ships, causing significant dangers to aquatic life. Because of the leaking of oil, including petrol, diesel, and other forms of hydrocarbons, from shipwrecks, mismanagement, and calamities, oceans have been discovered to be polluted with hazardous substances. When dirty water comes into touch with the soil, it pollutes it even more. The process of removing hazardous chemicals from the sea and soil is both difficult and costly. One of the most efficient ways of removing oil from soil and water and making them safe for aquatic and terrestrial organisms is bioremediation. Bacterial, plant, and fungal species are used in bioremediation techniques (Wang et al., 2008; Bahadure et al., 2013). There are three ways of cleaning up oil spills: physical, chemical, and biological. The biological approach, also known as bioremediation, is more beneficial than the physical and chemical methods since it saves time and money. Toxic chemicals are accumulated on the site via chemical processes, resulting in environmental contamination. Oil spills, whether they occur accidentally or on purpose, have a significant impact on environmental contamination. Oil spills from ships have long been recognized as a significant environmental risk. The spilled oil is thought to have mostly dispersed the habitat of marine animals, fish, and seabirds. The thick, sticky crude oil flows may immediately harm fish and marine species, seas, and coastal ecosystems and endanger human health over time (Tansel, 2014; Safiyanu et al., 2015). Because of its extensive usage, as well as the related disposal procedures and unintentional spills, crude oil pollution is relatively frequent.

8 Bacterial Bioremediation

Bacterial species such as *Pseudomonas* species are employed in bioremediation because they have the ability to break down hydrocarbons from gasoline and diesel, minimizing the impact of oil spills. *Pseudomonas alcaligenes* can break down polycyclic aromatic hydrocarbons, while *Pseudomonas mendocina* and *Pseudomonas putida* can eliminate toluene. The bacterium *Pseudomonas veronii* can destroy a wide range of aromatic chemical compounds. These oil-based chemicals are consumed by bacteria. They use the chemicals as substrates for metabolic processes. Oil spills may be cleaned up with these microorganisms, which are prevalent in soil and water bodies. Other bacteria that help with bioremediation include *Achromobacter*, *Flavobacterium*, and *Acinetobacter* (Atlas, 1981; Wang et al., 2008) (Fig. 3).

Toluene and other monocyclic aromatic hydrocarbons, such as benzene, toluene, and xylene, can be degraded by *Pseudomonas putida*. The production of diol and breakage of the aromatic ring, as well as the formation of diacids such as cis–cis muconic acid, occur when aromatic hydrocarbons are degraded by bacteria (Wang et al., 2008; Bahadure et al., 2013). Using bacterial species, many *Pseudomonas* species have the ability to breakdown hydrocarbons from gasoline and diesel, minimizing the impact of oil spills. *P. alcaligenes*, for example, can degrade polycyclic aromatic hydrocarbons, but *P. mendocina* and *P. putida* can degrade toluene. *P. veronii* is capable of degrading a wide range of aromatic chemical compounds. Bacteria consume these oil-based molecules and use them as substrates for metabolic processes. These bacteria may be found in large numbers in water bodies and soil, and they are capable of cleaning up oil spills. The process of bioremediation is

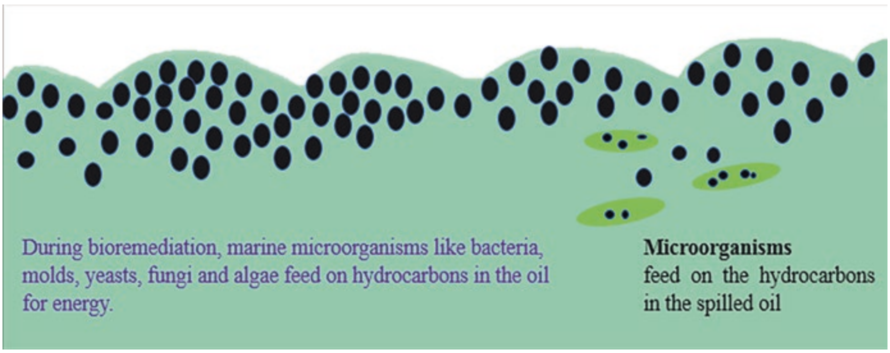

Fig. 3 Bioremediation processes by microbes

accelerated as the density of these microorganisms rises. *Acinetobacter*, *Flavobacterium*, and *Achromobacter* are some additional bacteria that aid in bioremediation. *Pseudomonas putida* is capable of degrading toluene as well as other monocyclic aromatic hydrocarbons such as benzene and xylene. The production of a diol followed by breakage of the aromatic ring and the generation of diacids such as cis–cis muconic acid is typical of bacterial degradation of aromatic hydrocarbons (Wang et al., 2008; Bahadure et al., 2013).

Algae, which are important components of the microbial community in both aquatic and terrestrial environments, have been found to participate in hydrocarbon biodegradation. Walker et al. (Walker et al., 1975) isolated *Prototheca zopfi*, an alga that can use crude oil and hydrocarbon substrates and degrades n-alkanes, isoalkanes, and aromatic hydrocarbons extensively. Cerniglia et al. (Cerniglia et al., 1980) looked at nine cyanobacteria, five green algae, two diatoms, one brown algae, and one red alga, to see if they could oxidize naphthalene.

9 Mycoremediation

It is the process of degradation of environmental toxicants with the help of fungi. In the natural world, fungi are the most significant decomposers. Fungi generate enzymes that aid in the degradation of cellulose and lignin. These two chemicals provide structure to plants and are extremely long-lasting. Fungi may also break down a few hazardous chemicals via similar processes (Wolski et al., 2012). It's used to clean up polluted soil, contaminated surface water, oil spills, industrial pollutants, and farm waste, among other things. *Lentinus edodes* (shiitake mushrooms) is an example of mycoremediation since it can breakdown pentachlorophenol (PCP), a broad-spectrum biocide that is more harmful than DDT (Fig. 4).

Pleurotus pulmonarius (Italian oyster mushroom) has capability to break down atrazine, a pesticide that contaminate groundwater in many Midwestern states. The white rot fungus, *Phanerochaete chrysosporium*, degrades chemicals like biphenyl and triphenylmethane (Wolski et al., 2012).

In the kingdom of Fungi, *Penicillium* species belong to the phylum Ascomycota. *Penicillium* species can be found in the air, on surfaces, and in food. *Penicillium chrysogenum* is generally present in salted meat, dried cereals, indoor air environments, salty soils, and marine water. *Penicillium* strains have been identified as the best hydrocarbon assimilates, with several publications demonstrating their capacity to convert xenobiotic chemicals such as phenol into less mutagenic products. Many companies that damage the environment create phenol (Atlas, 1981). Benzene, toluene, ethyl benzene, xylene, phenol compounds, and heavy metals including nickel, lead, and iron are all removed and degraded by the *Penicillium chrysogenum* strain. *Penicillium chrysogenum* and other fungi usually oxidize aromatic hydrocarbons and produce trans-diol (Pereira et al., 2014; Abdulsalam et al., 2012).

Fig. 4 Mycoremediation process by using mushroom

10 Phytoremediation

It is a new method that uses specific plant species to remediate various types of pollution in the environment, such as cleaning up groundwater and soils polluted with hydrocarbons and other harmful chemicals. Hydraulic control, phytovolatilization, rhizoremediation, and phytotransformation are all processes that may be utilized to remediate a wide range of pollutants.

For large sites with shallow residual levels of contamination by organic, nutrient, or metal pollutants, phytoremediation may be cost-effective if contamination does not pose an immediate danger and only “polishing treatment” is required, and vegetation is used as a final cap and closure of the site (Schnoor et al., 1995).

Phytoremediation has several advantages, including economic effectiveness, aesthetic benefits, and long-term application. Even then, using phytoremediation as a secondary or polishing in situ treatment stage reduces land disturbance and eliminates the transportation and liability costs of offsite treatment and disposal. Over the last 15 years, research and experimentation in phytoremediation for the treatment of petroleum hydrocarbons has yielded a lot of useful knowledge that can be used to design effective remediation systems and manage ongoing progress and innovation. Phytoremediation may be used to clean up a wide range of polluted areas. The possibility of using phytoremediation on hydrocarbon-contaminated areas was examined. The Alabama Department of Environmental Management approved a site with roughly 1500 cubic yards of soil and 70% of the baseline tests indicating total petroleum hydrocarbons above 100 ppm. Following a year of vegetative integument, over 83% of the samples had less than 10-ppm total petroleum hydrocarbon. Expulsion of total petroleum hydrocarbons from different field locations contaminated with petroleum refinery wastes, crude oil, and diesel fuel at initial TPH concentrations ranging from 1700 to 16,000 mg/kg has also been studied (Das & Chandran, 2011).

Plant growth has been discovered to be species-dependent. The presence of certain species resulted in a higher loss of total petroleum hydrocarbons than the absence of other species. Milo (*Thespesia populnea*), kou (*Cordia subcordata*), kiawe (*Prosopis pallida*), and the native shrub beach naupaka (*Scaevola serica*) have all survived field conditions and aided in the cleanup of diesel-polluted soils in the Pacific Islands (Kamath et al., 2004). When organic pollutants are present, the grass is frequently planted alongside trees as an initial remediation strategy. The presence of a large number of fine roots on the soil surface was discovered to have an impact on the binding and transformation of hydrophobic contaminants. Grasses are frequently planted between rows of trees to help stabilize the soil and guard against wind-blown dust, which can carry pollutants away. Some legumes, such as alfalfa (*Medicago sativa*), alsike clover (*Trifolium hybridum*), and peas, can help replenish nitrogen in depleted soils (*Pisum* sp.). Plants such as fescue (*Vulpia myuros*), rye (Elymus *sp*.), clover (*Trifolium* sp.), and reed canary grass (*Phalaris arundinacea*) are utilized successfully in many locations that have been contaminated with petrochemical wastes. The grass from these plants may be composted when they are harvested. It might be a very useful method for removing diesel-range organics from contaminated, vegetated soils (Miya & Firestone, 2001).

11 Use of Genetically Modified Bacteria for Bioremediation

The use of genetically modified microbes (GEMs) in bioremediation has got a lot of interest as a way to enhance the degradation of hazardous wastes in the lab. Genetic engineering technology has been used to revolutionize the bioremediation of hydrocarbon pollutants utilizing bacteria in numerous cases. The degradative effectiveness of the genetically engineered bacteria was greater. For effective in situ bioremediation utilizing genetically engineered bacteria, a combination of microbiological and ecological understanding, as well as biochemical processes, is required.

12 Conclusion

When it comes to cleaning up marine oil spills, bioremediation offers a lot of advantages over traditional physical and chemical approaches. One of the main benefits of bioremediation is that it is both cost-effective and time-saving when it comes to cleaning up a contaminated environment. When applied appropriately, the cost savings of bioremediation outweigh the disadvantages of standard cleaning methods. Bioremediation, unlike chemical techniques, does not require the use of harmful or foreign substances to clean up contaminated areas. Bioremediation does not cause the natural habitat environment to be disrupted, as physical and chemical cleanup approaches sometimes do. Bioremediation allows microorganisms to breakdown complex hydrocarbons into simple hydrocarbons with no negative environmental consequences. The world's major challenge in the subterranean environment is cleaning up petroleum hydrocarbons. By using different physical and chemical approaches, the microbial degradation process aids in the removal of spilled oil from the environment. It's possible because bacteria have enzyme systems that can breakdown and use various hydrocarbons as a carbon and energy source.

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