**Removal of spilled oil deposits through bioremediation**

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**ABSTRACT**

Oil spills have been occurring in the environment since the last century, mainly due to transportation and oil processing. It should be treated properly due to its environmental, economic and social threats. Several physical, chemical and biological methods are available to clean up the oil spills. Bioremediation is the most promising method for pollutant removal compared to the physicochemical method, especially after the Exxon Valdez oil spill. Bioremediation has several advantages over other methods, such as cost-effective and environmentally friendly technology. Both natural and genetically modified microbes can be applied to polluted sites with enriched nutrients. Bioremediation can be divided into bioattenuation, biostimulation and bioaugmentation based on the method followed to remove the pollutants. The researchers have carefully evaluated these methods for oil treatments in the laboratory and, to a lesser extent, in the field.

**Keywords—** Oil spill; bioremediation; Microorganisms; GEMs

**I. INTRODUCTION**

Oil spills occur when large quantities of liquid petroleum hydrocarbons leak into the environment from pipelines, non-suitable waste disposal, storage tanks, etc. [1]. It is estimated that 1.7 to 8.8 MMT of oil is released every year into the world’s water [2], of which >90% is directly related to anthropogenic activities. These include equipment and unit cleaning or accidents during transportation [3]. Most petroleum hydrocarbon spillage occurs during shipping, onshore and offshore exploration and transportation [4].

Oil is a group of related materials rather than a specific chemical compound extractable by certain solvents [5,6]. Oil compounds are nonpolar; thus, they are hydrophobic [6]. Under anaerobic conditions, oil hydrolyze into glycerol and long-chain fatty acids [7,8]. The glycerol further degrades into 1, 3-propanediol [9]and subsequently to acetate [10]. An oil-water mixture with droplets ≥ 150 µ is classified as free oil, while an oil-water mixture with a size ranging between 20 and 150 µ is classified as a dispersed oil mixture. The emulsified oil mixture is an oil-water mixture with droplet sizes smaller than 20 microns and an oil-water mixture with droplet sizes smaller than 5 microns are classified as a soluble oil mixture [11].

Oil spills can occur in terrestrial and marine environments and threaten human health and the ecosystem [12]. Oil spills pollute the water, risk explosion and cause fire, ruin the water and air quality, destroy the recreational area, and waste nonrenewable resources. The consequence of oil spills on the natural ecosystem is widespread and long-term. Marine oil spills, particularly large-scale oil spills, have received great attention due to their catastrophic damage to the natural ecosystem. In 1989, the spill of 37,000 metric tons of North Slope crude oil into the Prince William Sound, Alaska, from the Exxon Valdez led to the mortality of thousands of marine mammals and seabirds, a significant reduction in the intertidal and subtidal organisms and also causes many long-term environmental impacts [13]. Minor oil spills from non-point sources, such as urban runoff and boat bilges are less threatening to human health and the environment. Although minor oil spill has received much less attention in the past, the recent National Water Quality Inventory reports suggest that non-point source pollution remains the Nation’s largest source of water quality issues [5,14]. Oil spill occurrence is most common in sea and shoreline since the petroleum is usually transported through water. The mutagenic and carcinogenic effects of oil spills in the sea have been proven. Once the oil spill occurs, it prevents oxygen penetration and light diffusion in the bottom layers of the sea [15]. Oil spills in the ocean have a severe adverse effect on marine life and the environment. Therefore, there is a need for a trained workforce who can take suitable action shortly after an oil spill [16].

Several guidelines are available for oil spill prevention, management, and compensation. However, oil spills are inevitable, and the government should be prepared to take necessary action. Development of federal government blueprint (NCP), assigning a national authority, and response capability are the duties of governments [17].

Several conventional oil spill countermeasures include physical, chemical and biological methods. Commonly applied physical methods are manual removal, booming and skimming, sediment relocation, water flushing and tilling. Physical containment and recovery of the free oil is the primary option of the United States for the cleanup of oil spills in freshwater shorelines and marine environments. Chemical methods, especially dispersants, have been used in many countries. However, the chemical method has not been extensively used due to its toxicity, ineffectiveness and long-term environmental impacts [5]. The development of less toxic chemical dispersants may increase its potential application.

Although physical and chemical methods are often the first response option in oil spill removal, they rarely achieve complete cleanup of oil spills. Bioremediation is an emerging technology, particularly complete oil spill removal. The bioremediation approach was developed based on the principle that the oil components are readily degradable by microorganisms [18,19]. Bioremediation has several potential advantages over conventional methods, such as being less costly and more environmentally friendly. This article focuses on oil spill removal through bioremediation**.**

**II. SOURCES OF OIL**

 The largest source of oil is from oil mills. The oil mill effluents, such as palm oil mill effluent (POME), have 4000 to 6000 mg/l of oil [20].Untreated domestic wastewater contains 50 to 100 mg/l of oil concentration [21].Kitchen greywater is the highest contributor of oil to domestic wastewater [22].Food processing industries are well known for producing effluents containing oil. Unit cleaning, accidents during transportation and petroleum hydrocarbon spillage during shipping are the major oil source in the ocean [3]. Most petroleum hydrocarbon spillage occurs during shipping, onshore and offshore exploration and transportation [4].

**III. EFFECTS OF OIL SPILL**

 Oil spills have a major impact on local industries, human health and the marine ecosystem. Tourism, including fishing, swimming and sailing, will be affected until the water is cleaned up. Industries that rely on fresh seawater are also affected and cannot resume their activities until the water is cleaned up. The fishing industry suffers largely, not only because of the large amount of marine life lost and also because the catch obtained would be covered with oil and hence inedible as they are poisonous. Exposure of humans to oil spills may cause neurological, acute toxic effects, ocular, skin irritation, and respiratory system problems. People living in affected areas showed nausea, throat infections, migraines and headaches. Consumption of oil-contaminated seafood is dangerous as oil products have polycyclic aromatic hydrocarbons (PAH). These are human carcinogens. Once an oil spill occurs in the marine environment, it loses seagrasses and other vegetation. It is harmful as these are food supplies for most marine animals. Living coral is also vulnerable to oil slicks. Once the living coral dies, the reef of coral can be destroyed by wave erosion. This means many fish and animals lose their habitats.

Mangroves are salt-tolerant trees and provide habitat for sea birds, crabs, and oysters and serve as breeding grounds for fishes. Their complex root system prevents soil erosion. Mangroves are vulnerable to oil spills as they obtain oxygen through lenticels on aerial roots, also known as pneumatophores, which may become clogged by oil and stop the oxygen supply. Toxic components in oil may also upset plants mechanism of maintaining salt balance. The oil spills destroy the natural habitat of seaside animals like sea turtles. Sea turtles can be exposed to the oil spill as they stay ashore for the nest and when the oil floats over to beaches, it destroys the eggs. Sea birds are vulnerable to oil spill as it swims and dive into the water for their food and are most likely to be damaged. Even a small amount of oil can cause danger to the bird. The oil coating over the birds does not allow them to fly and thus destroys their natural waterproofing. The feathers of many seabirds are wettable and must be carefully dried for flight. If the feathers of seabirds come into contact with oil, the seabird ingests the oil while trying to preen. Filter-feeders, such as clams and oysters, intake surface water through their gills and filter it to take out any food. According to the reports, the Exxon Valdez oil spill killed approximately 2,50,000 to 5,00,000 seabirds [23]. The oil in the water gets concentrated within shellfish and then accumulates in their predators in a higher concentration; accordingly, the entire food chain will accumulate oil residues.

One of the most visually alarming physical effects on mammals is smothering, where oil coats the outside of their bodies, covering fur and creating a problem for the marine mammals to breathe. Toxic compounds from the spilled oil can be ingested by animals directly, by their prey, as they breathe and clean their fur, causing short and long-term negative impacts. The oil spill also causes a big danger to marine fish, which causes mortality as they are exposed to a large quantity of oil.

**IV. OIL SPILL REMOVAL STRATEGIES**

The faster response to the spill prevents the spread and contamination of oil in the environment [3]. The first important response to an oil spill is controlling the source of the spill and preventing the oil spread. This response can be any strategy or method to control the spill and its negative consequences. Several methods and treatments are available to countermeasure the oil spill, including physical, chemical, and biological methods [24]. The physical method includes booms, barriers and sorbents, dispersants, skimmers and *in situ* burning [17]. The common strategy used to control the oil spread with the barrier application and concentrate the oil into a thick layer by booms to enable the oil removal by different skimmers. The water is often filtered in the skimmer boats and sent back to the ocean while the oil is transferred to a holding tank. *In situ,* burning is another method that is followed to remove the oil contaminant from the ocean. After *in situ* burning, it is necessary to perform a toxicity assessment. This approach was followed in the Deepwater Horizon accident [23].

Dispersants are used in the chemical method, which is sprayed on the oil spill to break it up into small droplets to make it consumable by microorganisms more easily[23]. Common dispersants used for oil spill removal are Sulfonated naphthalene formaldehyde condensate (SNFC), sulfonated melamine formaldehyde condensate (SMFC), lignosulfonates, polyacrylamides.

Conventional methods can effectively remove oil spills but produce several hazardous compounds [24]. In contrast, biologicalmethods detoxify hazardous compounds and are less disruptive than excavation methods in the case of soil.

**V. BIOREMEDIATION OF OIL SPILLS**

Bioremediation aims to enhance the metabolic activity of microorganisms in the polluted sites and, consequently, stimulate the oxidation-reduction of the oil contaminants. In this method, microbes degrade the organic compounds of the contaminants [25]. This method was developed in the 1940s and gained more attention after the Exxon Valdez oil spill in the 1980s [26]. It requires more time for effective cleanup. Bioremediation is cost-effective, has no significant adverse effects, has simple technology and minimal physical disruption [27, 12]. The main disadvantage of this method is that a specific approach is needed for each polluted site and each type of spill. Microbes use petroleum hydrocarbon as a substrate to decompose pollutants into water, CO2, and other harmless compounds [28]. When bioremediation is used to treat an oil spill, some materials are added to the polluted environment. The materials may be the nutrients to enhance the growth of indigenous microbes or non-native microbes having enhanced the ability for hydrocarbon degradation were added to the contaminated environment. It is used as a complementary treatment after conventional cleanup [27]. It requires a longer period for effective cleanup, and in the case of a highly contaminated environment, bioremediation is less effective [29]. In the case of the shoreline oil spill, bioremediation is effective and faster. Whereas in the case of burned oil, materials such as microorganisms and nutrients should be added to enhance the cleanup [30].

The adaptation skills of the microbes and their resistance in the pollute environment is also an important factor [15]. However, even adapted microbes are not effective for bioremediation in a highly polluted environment [26]. Because during oil spills, the level of petroleum hydrocarbons goes beyond the tolerable limit of the microbes [31].

Different microorganisms such as bacteria, fungi, microalgae and yeast can degrade hydrocarbon pollutants. Bioremediation can be performed in two ways; i) in situ and ii) ex-situ [32]. In the ex-situ method, the contaminant matrix was extracted and treated elsewhere, while during in situ process, bioremediation occurs in the place of contamination [25]. In-situ approach is cost-effective and much safer than ex-situ [32].

**VI. ESSENTIAL FACTORS FOR MICROBIAL BIOREMEDIATION**

Several parameters, such as physical and chemical parameters, have a major impact on bioremediation. The physical parameters include temperature, pressure, and pollutant surface area and the chemical parameters are nutrient and oxygen availability, salinity, acidity, and pollutant nature and composition. Among them, most of the parameters can be manipulated to accelerate the natural bioremediation, while some of the factors, such as salinity, are not manipulated in the field [27].

Temperature is the major factor that impacts viscosity, solubility and toxicity. The rate of biodegradation increases with increasing temperature. The optimum temperature for biodegradation in seawater, freshwater and soil were 15-20 °C, 20-30 °C and 30-40 °C, respectively. Dissolved oxygen is also required for the degradation and oxidation of chemical contaminants. Usually, there is no oxygen limitation in the sea and freshwater. However, oxygen may be limited in some sediments and wetlands. In such cases, anaerobic degradation can be carried out. Uplow anaerobic sludge blanket (USAB) is the bioreactor system used in ex-situ bioremediation. The advantage of anaerobic degradation over aerobic is less space utilization and less or no energy requirements [33]. Pressure is also an important factor that can impact bioremediation. When the pressure increases, the rate of degradation decreases. The surface area of the pollutant can impact the oil and water interface. The rate of bioremediation increases with increasing surface area. At higher pH, the rate of petroleum hydrocarbon degradation increases [27].

**VII. TYPES OF BIOREMEDIATIONS**

Bioremediation can be divided into three types based on the method followed to remediate the pollution. They are bioattenuation, biostimulation and bioaugmentation. The bioattenuation (natural attenuation) process is based on the metabolic potential of microorganisms to detoxify or transform the contaminants. It is often considered as do-nothing solution, but it requires constant monitoring of the contaminant on the site. The time required for natural attenuation depends on site conditions and the specific contaminant. Natural attenuation in some sites may take a very long time because of its persistent nature and lack of appropriate degrading microorganisms.

Biodegradation can be stimulated by creating the correct environmental conditions for microorganisms. This process is known as biostimulation. There are two ways by which biodegradation can be stimulated; they are 1. supplying fertilizers (nitrogen and phosphorus), growth supplements and trace minerals; 2. providing environmental requirements like pH, temperature and oxygen to speed up their metabolism rate and pathway. All these activities are performed to accelerate of oil degrader’s growth and activity. This approach is also known as fertilization or nutrient enrichment [27]. Good results have been obtained by using this approach on sediments of the coast contaminated after the Exxon Valdez spill in Alaska, and the rate of biodegradation increased three to five times with the addition of fertilizers, such as iron, phosphorus, and nitrogen. It was observed that for crude oil degradation, the addition of nitrate is more effective than ammonia in seawater, while in salt-marsh soil, the addition of ammonia is more effective than nitrate [27].

In bioaugmentation, microorganisms with enhanced ability to degrade petroleum hydrocarbons are inoculated to the polluted site. This method is followed when the population of hydrocarbon-degrading microorganisms is low or there is a need to degrade a particular hydrocarbon that cannot be degraded by indigenous microbes (E.g., Polyaromatic hydrocarbon). There are 70 genera of microbes that are known to degrade hydrocarbons. Commonly the non-indigenous microbes from other polluted environments are used to be added to the target site [27]. Alternatively, microbes from the target site are separated, mass cultured under laboratory conditions in bioreactors, and used as inoculum to the target site. This process is known as autochthonous bioaugmentation, where bioremediation can be done by the native microbes of the contaminated site [26]. When the seeding is done by the enhanced indigenous organisms taken from the target site, the adaptation problem is avoided.

**VIII. MICROORGANISMS FOR BIOREMEDIATION OF OIL SPILLS**

More than 200 species of bacteria, fungi and yeasts are reported to degrade petroleum hydrocarbons. These organisms were naturally found in freshwater, marine and soil environments. Almost 79 bacteria, 9 cyanobacteria, 103 fungi, 14 microalgae and 56 yeasts were able to degrade petroleum hydrocarbons [34. 27]. Indigenous soil bacteria such as *Pseudomonas* strains isolated from soil and aquifers can degrade polycyclic aromatic hydrocarbons (PAHs) [4]. Other microbial species with the ability to degrade petroleum hydrocarbons are *Alcaligenes* sp., *Alcanivorax* sp., *Acinetobacter* sp., *Bacillus* sp., *Capnocytophaga* sp., *Cellulomonus* sp., *Corynebacterium* sp., *Dietzia* sp., *Enterobacter* sp., *Flavobacter* sp., *Gordonia* sp., *Microbulbifer* sp., *Micrococcus* sp., *Moraxella* sp. *Providencia* sp., *Roseomonas* sp., *Sphingobacterium* sp., *Sphingomonas* sp., *Stenotrophomonas* sp., *Streptococcus* sp., and *Yokenella* sp. [24].

Some fungi are also able to degrade pollutants. However, they require a longer time to degrade petroleum hydrocarbons effectively. Fungi belonging to *Amorphoteca* sp., *Penicillium* sp., *Aspergillus* sp., *Graphium* sp., *Fusarium* sp., *Talaromyces* sp., *Neosartorya* sp., and *Paecilomyces* sp. are capable of degrading petroleum hydrocarbons [35]. Yeast species, including *Candida* sp., *Pichia* sp., and *Yarrowia* sp., can also degrade oil compounds in the contaminants [24]. Some reports suggest fungi can degrade petroleum better than bacteria in certain circumstances. Still, there is a little information on fungal bioremediation in polluted marine sites [15].

Long-term exposure of microorganisms to a polluted environment leads to genetic selection. After adaptation, the microbes develop hydrocarbon catabolic genes in the plasmid and degrade hydrocarbons [32]. Microbes with increased oil degradation ability were observed in *Alcanivorax* sp. and *Cycloclasticus pugetii* [34]. Using indigenous microorganisms available in the contaminated sites is most appropriate for bioremediation as the indigenous microorganisms adapted quickly to the available environmental conditions. The main disadvantage of using indigenous microorganisms for bioremediation is the slow degradation rate and toxicity of some organic contaminants. This is severe in new man-made pollutants since the microbes have not developed any resistance mechanism for their degradation [36]. When the indigenous microbes failed to degrade hydrocarbons, oil-degrading microorganisms were added to the contaminated environment [27].

**IX. GENETICALLY MODIFIED MICROORGANISMS FOR BIOREMEDIATION OF OIL**

The first genetically modified microorganism (GEM) was developed in the 1970s. These microbes were named “superbugs” and can able to degrade oil [37]. Superbug was built in 1979 by Ananda Mohan Chakrabarty. The US government granted a patent for the construction and use of superbug. In 1990, the American Government allowed to use the superbug to clean up oil spills in Texas state water. Superbug is a multi-plasmid strain containing CAM-OCT-XYL-NAH plasmids capable of degrading camphor, octane, hexane, decane xylene, toluene and naphthalene. The construction of a superbug involves the following steps: 1. Identification and isolation of parent strains (*P. putida*) *that* could degrade camphor, octane, xylene and naphthene from polluted soils; 2. conjugative transfer of plasmids; 3. selection of superbug from this mixture of strains done by culturing the strains in the presence of all the four pollutants. Only superbugs can survive in that medium and it is subcultured in fresh medium lacking the pollutants for future use; 4. mass culture of superbug is done by either culturing in a suitable liquid medium in a large bioreactor or sprinkling over paddy straw and the straw is dried in the shade. The bacteria inoculated straw can be stored for over a year to treat oil spills.

The development of GEM became more popular in the early 1980s after the improvement of genetic engineering methods. In 1981, two GEM strains were developed and patented. They are *Pseudomonas aeruginosa* (NRRL B-5472) and *Pseudomonas putida* (NRRL B-5473). These strains contain the genes that provide the essential proteins to degrade camphor, salicylate and naphthalene [38]. Two operons available in these strains (xylUWCMABN and xylXYZLTEGFJQKIHSR) encoding enzymes involved in toluene metabolism m-ethyl toluene and m- and p-xylene. *P. fluorescens* HK44 was the first GEM that was approved to be used for bioremediation in the real field. This study was done to aim for long-term bioremediation of naphthalene-contaminated environment. This bacteria contains the plasmid, pUTK21 is made by inserting transposon Tn4431 into the NAH7 plasmid obtained from *P. fluorescens* 5R. Simultaneous degradation of naphthalene and the luminescent signal was due to the genes which promote the pathway for naphthalene decomposition and gene cassette (*lux*) [38].

Researchers at the University of Texas, Austin, revealed the genetic code of petroleum hydrocarbon degradation during the Deepwater Horizon oil spill in 2010. They have sequenced the DNA of the microbes that have oil degradation ability to uncover the genetic characteristics of several bacterial species. The construction of GEMs with an enhanced ability for biodegradation of organic compounds is possible since the degradative mechanism, the enzymes, and the relevant genes are understood and biochemical reactions are explained thoroughly. It was found that the ability of some bacteria for oil degradation is far greater than expected, especially for aromatic hydrocarbon (for example, Alcanivorax was formerly considered incapable of oil degradation [38].

For the proper development of GEM, there is a need to understand the breakdown mechanism of petroleum hydrocarbons, biochemical pathways, operon arrangement and genetic basis of interaction [37]. The major limitation of GEM is survival in the environment and public acceptance, which hinders their wider application in bioremediation [27], probable gene transfer to other microorganisms and co-release of antibiotic resistance markers, the concerns about the environment and public health safety limit the research with application of GEMs in real fields.

Several authorities are reluctant to accept the release of GEMs due to their adverse environmental impact, such as gene transfer. However, it must be noted that GEMs do not add new genes to the environment and are taken from another microorganism, and usually, the introduced engineered microorganism will not survive for a long time after exhaustion of its specific substrate. Some methods are available to reduce the potential risk of GEMs in the real field: 1. Using some genetic barriers that restrict the recombinant bacteria survival and gene transfer in the environment. The restriction can be achieved by kind of transposons free from transposase genes or by eliminating conjugation gens from a plasmid [38]; 2. A novel strategy is the construction of suicidal GEMs that can be achieved by adding antisense RNA and degradative operon to the construct. These novel GEMs make microbes susceptible to death after finishing the degradation of contaminants and reducing their risk to humans and the environment.

A successful application of GEM was used for the bioremediation of plants which was polluted by polychlorinated biphenyls. In this case, genetic engineering tools were applied to change the biphenyl dioxygenase enzyme of *Pseudomonas alcaligenes* KF707 and *Pseudomonas* sp. LB400 by modifying their substrate specificity. The substrate range of these microbes was combined, and various biphenyl dioxygenase enzymes were created that can oxidize double ortho- and double para-substituted PCBs [39].

**X. CONCLUSION**

Oil spill occurrence in land and water is not a new problem. The spill occurring in soil or water greatly threatens the natural ecosystem, flora and fauna. Compared with the physicochemical method of pollutant removal, biodegradation is a more effective and eco-friendly method without disrupting the natural environment. Although several researchers have studied this approach, a high hydrocarbon removal rate was observed in the laboratory, and the proper technology for field applications is yet to be developed. Bioremediation is an emerging technology that can be simultaneously used with other physical and chemical treatments to complete diverse environmental pollutants. It is a sustainable approach to environmental pollution management, and hence, there is a need for more research in this area. The investigation of GEMs application for bioremediation was done mostly in laboratory experiments. However, to understand the real effect of GEMs, long-term bioremediation in the real field must be done. This is necessary for determining the overall effectiveness and potential risk to the ecosystem.

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