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Review Article

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# Isolation and Characterization of Crude Oil Degrading Bacteria from Contaminated Soil

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**Abstract**

*In this modern world the need for petroleum products has highly increased for the contribution of modern lifestyles. Petroleum products are complex mixtures which are mainly derived from crude oil and they are processed in oil refineries. Gasoline and diesel are some of the petroleum products refined with high allocation of energy from the oil refineries. When organisms encounter levels of oil or its by products they may experience adverse effects. The severity of these effects depends on the type of oil, the quantity present, how the environment interacts with it and the health status of the organisms when exposed to it (Obire and Anyanwu, 2009). The shift in the economic base of coal to crude oil and petroleum products, more especially after WWII, greatly increased the volume of these commodities being transported across the high seas. Incidents of oil spills have become a common problem in environmental pollution that endangers biota of the contaminated region. Such incidents have become a global problem particularly in industrialized countries and developing countries like Nigeria. According to Best and Seiyefa, (2014), an average of 240,000 barrels of crude oil are spilled in the Niger delta every year, mainly due to unknown causes (31.85%), third party activity (20.74%), and mechanical failure (17.04%) (Best and Seiyefa, 2014).*

**Keywords:** Crude Oil, Molecular Characterization, Morphological Test, Biochemical Test, Oil Degrading Bacteria

## 1. Introduction

### 1.1 Remediation of Crude Oil

To remove the crude oil pollution, microbial assisted crude oil degradation has been under radar for a long time and the research has been intensified in recent years. The use of processes to break down oil is an encouraging method for cleaning up environments that have been contaminated with oil. This approach has been extensively studied over a period and research in this area has significantly intensified in times. The research has been focused towards finding sustainable ways to clean up contaminated environments the release of such petroleum products into the environment shows a great impact on biotic and abiotic communities of that environment if not treated at time. Bacillus cereus was identified as potential oil degrader which was isolated from Lebanese oil polluted soil and marine environment Acinetobacter calcoaceticus and Alcanivorax diesel lei were identified as potential crude oil degrader from Caspien Sea and Persian Gulf degraded 98% of crude oil [1,2]. The removing of some harmful organic compounds in the polluted sites by the biodegradation was done with the help of bacterial species. Bacillus megaterium, Bacillus cereus, Micrococcus luteus, Staphylococcus aureus, Lactobacillus acidophiles, Neisseria flaviscence and Cornybacteriumxerosis were identified as potential hydrocarbon degraders isolated from Hyderabad. The release of hydrocarbons into the environment, whether accidental or due to human activities, is the main cause for water and soil pollution. Many bioremediation technologies have been developed to remove these contaminants, as some biological treatments are cheaper than chemical and physical treatments and sometimes result in complete mineralization. Several technologies have been adapted to restore the harmony of nature by reclaiming the soils and seas contaminated by such undesired spillages. However, the hunt for cheaper and faster processes is always been done to create a significant progress. One such technology is using indigenous microorganisms to degrade the hydrocarbon pollutants and remediate the lost soil parameters.

### 1.2 Physical Method

Excavation of crude oil contaminated soil is the quickest and safe way but not a sophisticated and cheap method. The contaminated soil is removed and transported to appropriate landfill for the disposal. The samples are collected from bottom and sidewalls of the excavated area to check if the site is clean or not [3-5]. Another physical method is the washing of contaminated soil. Washing with organic solvents such as ethanol- water mixture and ethyl acetate-acetone-water mixture exhibited significant removal of hydrocarbons from the contaminated soil [6]. Soil washing does not only treat the oil contaminated soil but also remove the heavy metals from the soil. The efficiency of washing can be enhanced by the addition of surfactants. Studies showed that both artificial and natural surfactants are helpful in the removal of crude oil. Different surfactants remove different fractions of crude oil e.g. artificial surfactant sodium dodecyl sulphate (SDS) removed aliphatic hydrocarbons while natural surfactants saponin and rhamnolipid removed polycyclic aromatic hydrocarbons from the contaminated soil. This method no doubt is simple and efficient, however, it is very prolonged, time consuming and very costly. Transportation of contaminated soil to disposal site is another big problem. Surfactants might be dangerous due to their possibility of adhesion to soil particles.

### 1.3 Chemical Method

Chemical Methods Chemical oxidation is an efficient method to remove dangerous wastes from the soil at the oil spilled sites. The efficiency of this method strongly depends on the soil matrix. Fenton's reagent, a mixture of Hydrogen peroxide and Ferric ion, is used for chemical oxidation. Hydrogen peroxide is a strong oxidizing agent that generates hydroxyl ions during Fenton's reaction while ferric ion acts as catalyst. Hydroxyl ions are very powerful and effective agents that destroy the contaminants present in the soil demonstrated that removal of oil from sand at lower pH by using Fenton's reagent is much efficient than at natural pH or peat [7,8]. Another efficient oxidant that is used for the removal of crude oil from soil is ozone. It is easy to generate, store and handle for in situ treatment. Polycyclic aromatic hydrocarbons are more reactive with ozone in comparison o alkanes. Reactivity of poly aromatic hydrocarbons depends on the number of rings, heteroatoms presence or absence and alkylation level. Ozone also support microbial community present in the soil as it generates oxygen on its degradation, so it can be helpful in bioremediation method to aid microbial growth [9]. Chemical method is a quick way to treat contaminated soil, but chemicals may pose a serious threat to the nearby soil and living beings due to leaching or side reactions.

### 1.4 Biological Paper

Bioremediation is a traditional method that involves the use of living organisms (bacteria, fungi and plants) to degrade harmful substances present in the environment. Bioremediation of crude oil from the soil is very efficient, cheap and environmentally friendly solution. The effectiveness of this method is depended on hydrocarbon concentration, soil characteristics and composition of pollutants [10]. PAH are the most resistant and toxic group of soil pollutants present in the crude oil. PAH get trapped in the soil pores after they enter into the soil and retained by the soil matrix. So, their removal from the soil is very difficult. Bioremediation is the most suitable method to remove PAH from the soil as microbes and plant roots can access these tiny pores easily.

## 2. Bio Remediation

Bioremediation is the use of living organisms, primarily microorganisms, to degrade the environmental contaminants into less toxic forms. It uses naturally occurring bacteria and fungi or plants to degrade or detoxify substances hazardous to human health and/or the environment. The microorganisms may be indigenous to a contaminated area or they may be isolated from elsewhere and brought to the contaminated site. Contaminant compounds are transformed by living organisms through reactions that take place as a part of their metabolic processes (Vidal 2001). Indigenous microorganisms can utilize the total petroleum hydrocarbons of crude oil as source of carbon and energy and break them down to simpler non-toxic compounds such as $CO_2$ and $H_2O$. The bacterial groups known to degrade hydrocarbons include Pseudomonas, Macrobacteria, Alcanivorax, Microbulbifer, Sphingomonas, Micrococcus, Cellulomonas, Dietzia and Gordonia groups. Molds belonging to the genera Aspergillus, Penicillium, Fusarium, Amorphoteca, Neosartorya, Paecilomyces, Talaromyces, Graphium and the yeasts Candida, Yarrowia and Pichia have been implicated in hydrocarbon degradation. Also, microbes used recently for bioremediation processes are usually consortia as different microbial strains having the same function can actually create synergistic effects and treat the experimented site sooner than expected. Possibility of one species removing the toxic metabolites (that otherwise may hinder microbial activities) of the species preceding it. It is also possible that the second species are able to degrade compounds that the first are able to only partially degrade.

Therefore, the present study also focuses bioremediation of contaminated sites using microbial consortia. The isolation and characterization of crude oil degrading bacteria for bioremediation purposes is essential for improving the efficiency and effectiveness of bioremediation. The characterization can also lead to development of specialized microbial enzymes or biofilters that can enhance the efficiency of crude oil biodegradation in the bioremediation process. The isolation and characterization step is also essential as it allows discovering efficient crude oil degrading bacteria, leading to the development of targeted bioremediation approaches. Many studies have documented successful bioremediation using oil-degrading bacteria. In recent studies, it has been seen that there is an abundance of PAH degrading bacteria present in crude oil contaminated environments that decrease PAH levels by bioremediation. Multiple genera of bacteria can degrade crude oil and are sampled from diverse kinds of sources. Such bacteria can also be isolated from non-contaminated sources. Various members of the families Alcanivoracaceae, Rhodobacteraceae, Rhodospirillaceae, Halomonadaceae, Oceanospirillaceae, Pseudomonadaceae, and Shewanellaceae have been reported to degrade oil and produce BS in enrichment cultures and contaminated areas. There are multiple methods to isolate or characterize crude oil degrading bacteria. Minimal Salts Medium (MSM) is usually used to isolate crude oil degrading bacteria. Linda U. Obi and her colleagues did molecular characterisation of the 16S rRNA genes and it was indicative that the isolates obtained on a mineral salt's medium belonged to different genera like Stenotrophmonas, Pseudomonas, Bordetella, Brucella, Bacillus, Achromobacter, Ochrobactrum, Advenella, Mycobacterium, Mesorhizobium, Klebsiella, Pusillimonas and Raoultella. However, the research shows that the media prefers isolating bacteria with biosurfactant properties. Other media such as Bushnell Haas Media (BHM), Nutrient broth, Trypticase soy broth, Marine broth can also be used for isolation. Some researchers have also developed in-house media which allows isolation of uncultivable bacteria. Liebeke and his colleagues developed a specialized media called Intensive Soil Extract Media (ISEM) made from soil extract that was able to isolate previously uncultivable bacteria present in soil.

## 3. Products Produced by Bacteria that Aid in Crude Oil Degradation

There are two major components produced by oil degrading bacteria that aid in biodegradation of crude oil components.

### 3.1 Biosurfactant

Biosurfactant are low weight amphiphilic compounds produced by microorganisms. These compounds have the ability to accumulate between fluid phases, thus reducing surface and interfacial tension at the surface and interface respectively. There is a growing interest in biosurfactants because of their low environmental impacts, low toxicity, and biodegradability. These compounds generated by living surfaces can reduce interfacial tension and surface tension. Genera Pseudomonas, Bacillus, Sphingomonas, and Actinobacteria include the foremost biosurfactant-producing bacteria. broth can also be used for isolation. Some researchers have also developed in-house media which allows isolation of uncultivable bacteria. Liebeke and his colleagues developed a specialized media called Intensive Soil Extract Media (ISEM) made from soil extract that was able to isolate previously uncultivable bacteria present in soil.

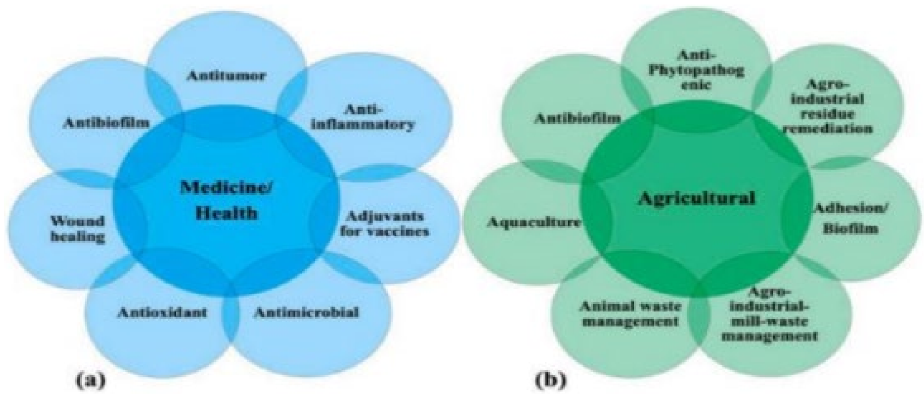


**Figure 1: Application of Biosurfactants in Medical and Agricultural Sector**

| Organism name | Bioemulsifier | References |
|---|---|---|
| *Pseudomonas sp.* | Ornithine lipids | *(J. D. Desai & Banat, 1997)* |
| *Pseudomonas fluorescens* | Lipopeptide | *(Neu et al., 1990)* |
| *Pseudomonas fluorescens* | Trehalose Lipid-o-alkyl, monoglyceride protein | *(K. M. and D. Desai J. D. ,. A. J. and Patel, n.d.)* |
| *Bacillus subtilis* | Subtilisin | *(Sutyak et al., 2008)* |
| *Bacillus licheniformis* | Lichenysin | *(Yakimov et al., 1995)* |
| *Bacillus subtiliss* | Surfactin | *(Arima et al., 1968)* |

**Table 1: Some Example of Biosurfactant Producing Bacteria**

### 3.2 Bio Emulsifier

These are high molecular weight amphiphilic compounds that are produced by various microorganisms. These are generally complex mixtures of heteropolysaccharides, lipopolysaccharides, lipoproteins, and proteins. A bioemulsifier has the ability to reduce surface tension and interfacial tension. As compared to chemical surfactants, the biosurfactants are more efficient and eco friendly. Due to their dual lipophilicity and hydrophilicity. They can create emulsions in either oil-in-water (O/W) or water-in-oil (W/O).

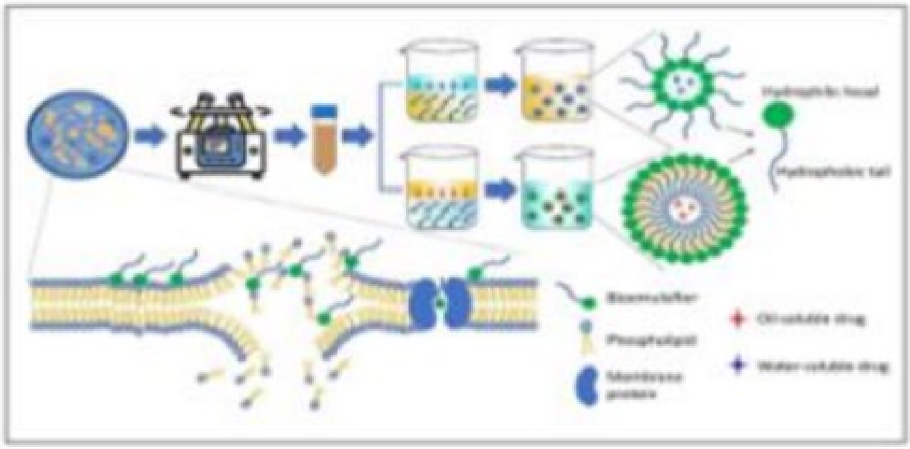

**Figure 2: Mechanism of Action of Bio Emulsifier**

## 4. Effect of Crude Oil Contamination on the Soil

The effect of crude oil on the soil depends on the size, quantity and grade of oil spilled. Crude oil spillage decreases the porosity of the soil. This is due to the fact that oil tends to force the soil particles to stick together, thereby decreasing the pores. Higgins and Burns (1979) reported that in oil-contaminated soil, oil droplets interfere with the interstices. Additionally, crude oil forms a coat covering the soil surface to retain carbon dioxide from the respiration of soil organisms. Persistence of oil on/in the soil depends on the amount spilled, procedures of clean-up, microbial degradation, climatic conditions, and the type of oil spilled. Crude oil changes the characteristics of the land, polluting it to the detriment of living organisms. Vegetation, wildlife, crops and farmlands are adversely affected. Toxicological studies have identified Polycyclic Aromatic Hydrocarbons (a derivative of crude oil) as being carcinogenic and have been implicated to be the cause of rapid death of living organisms. Oyefolu and Awobajo (1979) reported that a good percentage of oil spills that occurred on dry land between 1978 and 1979 in Nigeria affected farms in which crops such as rice, maize, yams, cassava, and plantain were cultivated. No permanent damage is however done to the soil except in cases in which the soil is completely submerged by the oil in areas of poor drainage and aeration. With volatilization, scientific principles, microbial degradation, rainfall and aeration, light oil spillage can be cleaned up within 2 to 3 years. Klokk (1984) studied the effect of crude oil pollution on the germination and vegetative growth of five species of vascular plants and reported a reduction in overall germination rate. Germination response to oil varied greatly with plant species and members of the same plant species showed differential sensitivity to oil contamination. Amakiri and Onofeghara (1984) reported that the seeds of Zea mays exhibited no germination after exposure to oil for longer than seven days while those of Capsicum frutescum exhibited 100% viability after 32 weeks exposure. Some adverse effects of oil spills and contamination of birds and aquatic animals have been documented. Chronic marine pollution in South Eastern Newfoundland (Canada) waters was reported to have led to the death of 74% of the sea birds that died between 1984 and 1999. The dead birds were found with oil on their feathers. An oil tanker accident involving the oil tanker Prestige in November 2002 where about 63, 000 tons of heavy oil reached the Galician Coast (NW Spain) was found to have caused different levels of DNA damage on birds exposed to the spill. Further studies on the impact of the Prestige oil spill and its clean up activities on the macroinfauna community of the Galician Coast six months after the spill, revealed that the macroinfauna population was drastically reduced, with Eurydice and Scolelepsis squamata as the most affected taxa evaluated the effect of diesel oil on aquatic species (Oncorhynchus mykiss and Daphnia magna) using acute toxicity testing and found that their mortality rates were significant compared to species not exposed to diesel oil.

## 5. Conclusion

Crude oil contamination is a very serious problem affecting both the terrestrial and aquatic environments. Its impact, especially on the coastal regions has been a source of concern to many people and governments of the world. The need to devise sustainable clean up methods have never been so crucial. This review has tried to highlight the different methods used for the clean up of crude oil contaminated soil. The choice of clean up method should depend on a number of factors which include, how well the soil is cleaned, the ability of the soil to be reused after clean up and how environmentally friendly the clean up method is. Other factors to be considered include the overall cost of the clean up exercise and also whether the clean up method is approved by the Environmental Protection Agency. Lodolo reported some other criteria to be considered in choosing a soil remediation technology to include the technique's ability to clean-up to a desired level (minimum pollutant concentration achievable by the technology), community acceptability, post-treatment costs, soil quality required after the intervention, environmental impacts and risks of remediation activities/processes. Observed that no single specific technology may be considered as a panacea for all contaminated site problems. There are also limitations involved in the use of each clean-up methods. Low permeability, high content and soil heterogeneity limit the performance of the SVE method. There is also the risk of potential release of hazardous compounds during excavation and materials handling. The major disadvantages of the thermal methods (incineration and thermal desorption) are the high energy costs involved and the danger of environmental pollution. The biological methods have been judged as the best remediation methods in terms of efficiency and environmental acceptability. Biological techniques are encouraged because of the advantage of soil sustainability and possibility of the soil to be restored to its original use. EPA has chosen bioremediation as a primary reasonable remedy to treat organic contaminants in soil, sludge, and sediments at wood treating sites [11-18].

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