Chapter

Biotechnological Potentials of Microbe Assisted Eco-Recovery of Crude Oil Impacted Environment

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Abstract

Globally, the environment is facing a very challenging situation with constant influx of crude oil and its derivatives due to rapid urbanization and industrialization. The release of this essential energy source has caused tremendous consequences on land, water, groundwater, air and biodiversity. Crude oil is a very complex and variable mixture of thousands of individual compounds that can be degraded with microbes with corresponding enzymatic systems harboring the genes. With advances in biotechnology, bioremediation has become one of the most rapidly developing fields of environmental restoration, utilizing microorganisms to reduce the concentration and toxicity of various chemical pollutants, such as petroleum hydrocarbons, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, phthalate esters, nitroaromatic compounds and industrial solvents. Different remediation methods have been introduced and applied with varied degrees of success in terms of reduction in contamination concentration without considering ecotoxicity and restoration of biodiversity. Researchers have now developed methods that consider ecotoxicology, environmental sustainability and ecorestoration in remediation of crude oil impacted sites and they are categorized as biotechnological tools such as bioremediation. The approach involves a natural process of microorganisms with inherent genetic capabilities completely mineralizing/degrading contaminants into innocuous substances. Progressive advances in bioremediation such as the use of genetically engineered microbes have become an improved system for empowering microbes to degrade very complex recalcitrant substances through the modification of rate-limiting steps in the metabolic pathway of hydrocarbon degrading microbes to yield increase in mineralization rates or the development of completely new metabolic pathways incorporated into the bacterial strains for the degradation of highly persistent compounds. Other areas discussed in this chapter include the biosurfactant-enhanced bioremediation, microbial and plant bioremediation (phytoremediation), their mechanism of action and the environmental factors influencing the processes.

Keywords: environment, bioremediation, phytoremediation, genetic engineered microorganisms, crude oil

1. Introduction

One of the major environmental problems facing industrialized nations in recent times is hydrocarbon contamination resulting from oil and gas exploration and exploitation activities. As the demand for liquid petroleum increases, the release of this essential energy source into the environment becomes inevitable and has caused devastating consequences to marine/coastal waters, shorelines and land as well. Human activities such as accidental release of petroleum products, uncontrolled landfills, sabotage, leaking of underground storage or improper storage of crude oil are of particular concern in the environment. Hydrocarbon components have been known to belong to the family of carcinogens and neurotoxic organic pollutants which constitutes a major health challenge globally. Oil spill on land, penetrates to a depth of about 10–30 cm and sometimes beyond, results in the loss of soil fertility and also initiates environmental degradation [1]. This consequently alters the physicochemical properties of the soil making it impossible for the soil to produce at its optimal capacity.

The application of biotechnology today as a tool for environmental clean-up has been widely studied. Biotechnological tools in eco-restoration of crude oil impacted sites involves the use of biological agents to decontaminate/detoxify, mineralize, transform or degrade toxic/harmful substances into innocuous forms. The process known as bioremediation is genetically-driven, whereby microbes with inherent enzymes harboring catabolic genes utilize these xenobiotics as a source of carbon and energy thereby decontaminating the environment. The biological agents in bioremediation; microbes (microbial bioremediation), plants (phytoremediation) or plant-microbe interaction and their mode of operation will be extensively discussed in this chapter. Nigeria and some other nations in Africa have experienced devastating consequences of pollution in all environmental compartments which till date is still a major challenge [2].

With the advances of biotechnology, bioremediation has become one of the most rapidly developing fields of environmental restoration, utilizing microorganisms to reduce the concentration and toxicity of various chemical pollutants, such as petroleum hydrocarbons, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, phthalate esters, nitroaromatic compounds, industrial solvents and the very recalcitrant substances [3]. This has been made possible through a very important, emerging and next generation approach, called genetic engineering which involves the modification of the genetic structure of an organism to increase/enhance their activity. This approach is one potential key to a very successful, and swift bioremediation, whereby the catabolic potentials of an organism is enhanced by the introduction of hydrocarbon catabolic genes into the microbe. This paper highlights the various biotechnological tools that can be practically adopted especially in Nigeria and Africa at large to encourage environmental sustainability and eco-restoration of crude oil polluted environments.

2. Crude oil pollution and environmental consequences

2.1 Crude oil as an environmental pollutant

The intensification and rapid increase of manufacturing industries and the intensive use of fuels has led to an increased release of a wide range of xenobiotic compounds to the environment. Overtime, continuous loading of excess hazardous waste and xenobiotic compounds into the water bodies and soil has led to the destruction of soil structure, component and biodiversity, scarcity of clean water thereby limiting crop production [4, 5]. One of the major types of pollution that have caused so much harm/damage to the ecosystem generally is crude oil pollution. Crude oil contains so many toxic compounds such as hydrocarbons which can be easily converted to activated metabolites or free radicals during their oxidation [6]. The high toxicity of crude oil is usually attributed to its low molecular weight hydrophobic petroleum hydrocarbons. Other larger constituents of crude oil include alkyl PAHs with three or more rings which are less soluble in water [7]. In the past, several incidents have occurred which caused devastating damage to the ecosystem and have revealed the importance of preventing the escape of effluents into the environment, one of such incidents is the Exxon Valdez oil spill [8]. The Exxon valdez, a cargo ship carrying crude oil was grounded on the 24th march, 1989 along the Bligh Reef in Alaska, northeastern Prince William Sound. This resulted in the release of about 20% of the entire cargo (about 36,000 metric tons) [9]. Another significant oil spill that occurred in the Gulf of Mexico in 2010 is the BP Deepwater Horizon spill. Approximately 4 million barrels of crude oil spilled from the Macondo Wellhead (MW) making it the largest accidental marine oil spill in history. The biological impacts of the oil spill were severe, including in the deep sea, a habitat typically characterized by high biodiversity and generally economic and ecological impact [10].

In Nigeria, crude oil and gas production contributes to 25% of the nation's gross domestic product (GDP) and about 90% of the foreign exchange. The exploration and production of crude oil has caused devasting impact to all environmental compartments within the country, especially in the Niger Delta Region [11, 12]. A constant reoccurring phenomenon is the leaks from oil tankers and petrol leakage into the soil and these slicks formed contribute to reduction of dissolved oxygen and co-marine environment which causes oil slick. Polycyclic aromatic hydrocarbons (PAHs) which are one of the major components of crude oil have been found in water ways as a result of pollution caused by the effluents from petrochemical industries. Some of the major activities that cause petroleum hydrocarbon pollution of the environment are oil well drilling production operations, refining, storage, transportation, marketing in the upstream and downstream industry, anthropogenic sources [13]. Some of the causes of oil pollution may also occur in form of spillages due to corrosion of pipelines, oil well blowout, vandalization of pipelines or accidental discharges.

2.2 Environmental consequences of crude oil pollution

Crude oil pollution has been reported to cause devastating environmental consequences. Its effects range from the destruction of the soil structure and biodiversity, to limitations in plant growths which may further affect the farmer's source of income, and health hazards in man. It has also been reported that plants that grow in oil polluted soils show signs of chlorosis on their leaves and are also retarded due to the water deficiency. These have led to a complete halt in some farming activities like fishing or even death in some cases when contaminated water or food crop is consumed [14, 15].

There are countless literatures on the study of the causes and effects of petroleum hydrocarbon contamination on human health, soil, plant growth and the environment in general [16–18]. Ojimba [19] conducted a research to determine the effects of crude oil pollution on crop production. He analyzed data from 17 out of the 23 Local governments in Rivers state, Nigeria. His results showed there has been a significant reduction in the size of available farmlands due to crude oil pollution, this further reduced the physical farm products by 1.09016 tons. His results also indicated that 78% of farm lands had less than 80% efficiency due to crude oil contamination. The study concluded that crude oil pollution on farmlands and crops has negative effects on the output of crops. Abii and Nwosu [20] reported that Crude oil pollution causes reduction in the fertility of the soil such that the major essential nutrients necessary for the plants to grow are almost completely lost. Other effects of crude oil pollution on plants may range necrosis, chlorosis, yield reduction, bleaching, spotting of leaves, malformations to mesophyll cells and epidermal layers [21].

Al-Qahtani [16] investigated the effects of sludge from oil refineries on soil properties and the rate of plant growth. He carried out the experiment by applying the refinery sludge in a plant *Vinca rosea (Catharonthus roseus).* The results showed that with increase in the application of the sludge, the soil chemical composition showed a reduction in dry matter yield and decrease in plant yield significantly. There was also increase in soil salinity with the application of oil refinery sludge. With the continuous introduction of the sludge, there was a significant decrease in the essential mineral elements of plants such as phosphprus and nitrogen compared to the control treatment. Ibemesim [22] conducted an experiment on the tolerance of sour grass (*Paspalum conjugatum Bergins*) in a crude oil polluted system. In their results, the crude oil polluted soil did not have any significant effect on the major growth parameters. Their result showed that polycyclic aromatic hydrocarbons (PAHs) was able to modify the absorption, uptake and availability of sodium ($Na+$) in the plant.

Sun et al. [18] conducted an experiment to study ability of the eggs and larvae of a marine medaka (*Oryzias melastigma*) to survive in crude oil polluted environment. The experiment was carried out by treating the eggs and larvae with three different treatments. The first treatment was with CO_2, the second was with a water-soluble fraction of crude oil which was prepared using crude oil and sea water in a volumetric ratio of 1:100 respectively and the third was mixed with a CO_2/water soluble fraction of crude oil mixture. The combined treatment (CO_2 and water soluble fraction) had no detectable effect on the size or survival rate, however there were significant anomalies of the tissues in treatments with the water-soluble fractions of crude oil. They concluded that crude oil pollution has the ability to perform as a contributory factor to natural mortality. Agbogidi et al., [23] carried out a study to examine the environmental and socio-economic impacts of oil exploration in two oil producing communities in Delta state, Nigeria. The study showed that crude oil spillage due to oil and gas industry activities (exploration and production) caused damages to arable soils and water bodies which have led to a reduction in crop yield and hence the income capacity of the farmers. The results also showed a heightened deforestation and increased health hazards due to the crude oil activities in the communities.

Obire and Anyanwu [24] also conducted an experiment to investigate the Fungal population at different concentrations of crude oil pollution in a soil sample. Their analysis showed high significant difference between the control and the oil treated soils, the total fungal counts of petroleum-utilizing fungi were relatively higher. Some of the fungi species isolated from the soil were *Candida, Alternaria, Mucor, Rhodotorula, Penicillium, Saccharomyces, Trichoderma, Rhizopus,* etc. they concluded that high concentration of crude oil has a significant adverse effect on the fungal population and diversity. These are effects of crude oil pollution in the ecosystem. They further recommended that this harmful effect justifies the need for bioremediation.

3. Biotechnological tools in eco recovery of crude oil polluted soil

Biotechnology is defined as the set of scientific techniques that makes use of biological systems or living organisms to make, modify or improve products which may be products mays be plants or animals [25]. It has also been defined as a process which involves developing organisms for specific purposes and it includes the use cell fusion, recombinant DNA and other novel bioprocess technologies [26]. Biotechnological tools in eco-recovery of crude oil polluted sites are those biotechnological processes that involves the use of bio-products and also microbes for production of environmentally friendly products, reduces pollution and its effect, and all general restoration and maintenance of the environment to its pristine (natural) state for the benefit of man and the environment [2]. Biotechnological tools in eco recovery are also concerned with prevention of processes capable of causing an unsustainable environment for man and eco-components. There are no known number of bio tools used in prevention or restoration of a polluted environment, however the most successfully applied, eco-friendly and cost effective tool in environmental decontamination is bioremediation. The different types of bioremediation (biosurfactant-enhanced bioremediation, microbial bioremediation, plant bioremediation, genetically modified microorganisms in bioremediation), mechanism of action and factors influencing the process will be discussed in this chapter.

Before now, remediation of contaminated/polluted environments have been carried out using conventional methods such as to cap and contain the contaminated areas of a site or digging up contaminated site and removing it to a landfill. These methods have however had some drawbacks. The first method is however just a temporary solution as the contaminants may still linger on the site and may further require monitoring and maintenance in the future, this leads to increased cost. In the landfill method, the contaminated soil is excavated moved to a different site and the excavation and transporting of the contaminants may pose a serious environmental risk, it might also prove expensive to find new sites for the disposal of the contaminated soil [27]. These drawbacks have led to the search for a better approach which would include transforming the pollutants to a harmless substance or a complete destruction of the pollutants if possible [28].

The use of biotechnology which entails the application of genetic modifications to improve the ratio of work done and reduce cost associated with remediation and eco restoration process have become a major factor for the increased exploitation of biological systems in waste reduction and eco restoration. Due to the urgency in the need for an effective and efficient biotechnological process and the need for a process that completely destroys the pollutants, researchers have come up with a technique for rehabilitating either contaminated sites or sites that have been degraded due to anthropogenic activities and the mismanagement of the eco system. This process is called bioremediation and it involves the application of living microorganisms to degrade environmental pollutants or to prevent pollution. The different strategies/tools used in bioremediation of oil spills include bio-stimulation, bio-augmentation, use of genetically engineered microbes, nutrient application, seeding with competent or adapted hydrocarbonoclastic bacteria or their consortium. Some of these Environmental biotechnological tools for the clean-up of crude oil contaminated sites are highlighted here.

3.1 Bioremediation

Bioremediation has been defined as the process of removing toxic waste from the environment using biological agents. According to Kumar et al. [27]. It was defined as the most effective tool to manage waste polluted environment and recovery of contaminated soil. Bioremediation have been carried out both in situ and ex situ in several sites around the world with very successful outcomes. This method is considered a non-invasive, cost effective and sometimes logistically favorable clean-up technology which attempts to accelerate the naturally occurring biodegradation of contaminants through the optimization of the prevailing conditions [29, 30]. Bioremediation alongside natural attenuation have provided solution for emerging contaminant problems using actions such as biological carbon sequestration, landfill stabilization, endocrine disrupters and mixed waste biotreatment. Plants and microorganisms play roles in the remediation of contaminated environment; thus, the purpose of bioremediation is to reduce the potential toxicity of chemical contaminants in the environment via degradation, transformation, and immobilization of the undesirable compounds through the introduction of biosystems such as higher organisms like plants (phytoremediation), microbes, and animals. Some of the microbes involved in bioremediation process may include aerobes, anaerobic bacteria, fungi and other microbes with degradative potentials. Several microorganisms including *Mycobacteria, Pseudomonas*, *Bulkhoderia*, *Enterobacter, Acinetobacter, Alcaligenes*, *Bacillus*, *Proteus* various *Corynebacteria* and some yeasts have been confirmed to degrade or utilize oil as a source of food [12, 31, 32]. Papadaki and Mantzouridou [33] reported that microorganisms involved in bioremediation are capable of converting or degrading contaminants such as crude oil that can be used as energy source. Microorganisms can also adapt to the stresses caused by pollutants in the environment due to some characteristics such as metabolic potentials that are inherited during natural selection or resistance to the pollutants and this contributes to the recovery and soil restoration process [34]. In a report by Adetutu et al. [35] microbes were able to remediate up to 46.6% of the oil in a contaminated soil in 320 days.

Several bioremediation strategies have been explored for treating different sites but most have been designed for land oil spill control. These strategies attempts to increase the efficiency of natural attenuation process and they include: landfarming, composting, use of bioreactors, bioventing/biosparging, pump and treat, bioslurping, biostimulation, and bioaugmentation [36]. A description of the in-situ and ex-situ bioremediation techniques is presented in **Table 1.**

3.1.1 Biosurfactant-enehanced bioremediation

Many microorganisms involved in bioremediation produce potent surface-active compounds that can emulsify oil in water called biosurfactants and unlike chemical surfactants, the microbial emulsifier is biodegradable and non-toxic thereby facilitating the removal of hydrocarbon pollutants especially in the marine environment [43]. Biosurfactants can improve hydrocarbon bioremediation by two methods; the first incorporates the increment of substrate bioavailability for microorganisms, while the other method includes interaction with the cell surface which builds up the hydrophobicity of the surface allowing hydrophobic substrates to relate more effectively with bacterial cells [44]. By bringing down surface and interfacial tension, biosurfactants causes an increment to the surface areas of insoluble compounds prompting expanded portability and bioavailability of hydrocarbons. In outcome, biosurfactants upgrade biodegradation and removal of hydrocarbons. Biosurfactants are known to increase biodegaradation of highly hydrophobic compounds such as aromatics, alkanes, resins, cycloalkanes [45] by increasing bioavailability of the hydrophobic compound through facilitated transport of the pollutants from the solid phase (such as communication between surfactants and hydrocarbons, communication between contaminants and single biosurfactant molecules), improvement on the apparent solubility of the contaminants (improve the apparent solubility of the hydrophobic organic compound), and emulsification of non-aqueous phase liquid contaminants (in this process biosurfactants can lower the surface tension between non-aqueous and aqueous phases, this then leads to an increase in improving mass transport, the contact area, and mobilization liquid-phase contaminants).

Technique/definition	Potential success	Limitations	Applicability	Reference
Landfarming: this involves periodic mixing of the hydrocarbon polluted soil for aerobic microbial degradation to occur	This process has been useful in degrading a number of hydrocarbon compounds. Suitable for treating large volumes of contaminated soil	Large amount of land is required Unsuccessful in degrading high molar mass PAHs It is a very slow .biodegradation process	Volatile organic compounds and light weight PAHs. It can be applied in-situ and ex-situ.	[37]
Bioventing/Biosparging: it is designed for the decontamination of hydrocarbons at the saturated and unsaturated zones with the supply of nutrients (if required) and oxygen.	Little disturbance to site operations, treatment time from 6 months to 2 years. Hydrocarbons can be degraded in both saturated and non-saturated zones	Too slow in degrading heavy fractions of PAHs. Can only be used where bio- sparging/bioventing is suitable. Absence of other natural processes involved in degradation	Saturated and unsaturated zone. Mid-weight and low weight petroleum hydrocarbons. In-situ bioremediation system.	[36, 38]
Composting: It utilizes biological agents in organic amendments to aerobically degrade spilled pollutants.	High oleophilic microbial population derived from the organic amendment and elevated temperature optimal for degradation of the pollutant. Produces an end product of mature compost suitable for agricultural purposes. Suitable for treating large volumes of soil.	Longer treatment time compared to other ex-situ techniques.	Stimulates hydrocarbon degradation and enhances availability of hydrocarbon pollutant.	[39]
Use of bioreactor: it comprises a bioreactor system that controls the environmental /nutritional factors that influence biodegradation.	Rapid degradation kinetics. Optimized environmental parameters. Enhances mass transfer. Effective use of biostimulants.	Excavation of polluted soils or pumping of contaminated groundwater to the treatment site that is cost-ineffective. Production of toxic sludge as a by-product of the bioreactor. Increased operational cost.	Containment of volatile organic compounds (VOCs) or polluted air emissions. Highly efficient in diesel and PAH degradation.	[40, 41]
Pump and treat: this system is specially designed to treat groundwater pollution by pumping the polluted water to this for treatment before re-injection	Encourages biodegradation of contaminants in the unsaturated zone. Effective groundwater clean-up technique. Cost intensive.	Location of the groundwater contaminant plume, designing a capture mechanism and installing extraction and injection wells		[42]

Table 1.
The most applicable in-situ and ex-situ bioremediation techniques for hydrocarbon removal.

Biosurfactants may be secreted outside the cells (extra-cellular) or located inside the cells (intracellular) [46]. Based on myriads of documented reports available on bacterial bio-surfactants, it has been established that the spectrum of activity depends on the chemical composition of the pollutant. A strain of *Pseudomonas aeruginosa* was reported by Patel et al. [47] to produce the rhamnolipid type of bio-surfactant which was mono as well as di-rhamnolipid. Rhamnolipid and its producing microorganism has been implicated in the specific degradation of hexadecance which clearly shows that there is a strong relationship between the type of surfactant and the type of hydrocarbon/oil that gets degraded. In another related study, a group of bacteria producing glycolipids and sophorolipids significantly degraded polycyclic aromatic hydrocarbons. Chakrabarti, [48] reported that in the presence of glycolipids Surface active glycolipids when introduced in to the hydrocarbon polluted environments have improved the biodegradation of 2,4-DCPIP.

Bacteria produce biosurfactants in the form of biofilm which interacts with an interface and alters the surface properties such as wettability and other properties. Biosurfactant producing bacteria have been to be isolated from different environmental compartments including the marine environment. A marine bacterium, *Pseudomonas aeruginosa* was isolated from sea water polluted with oil. This organism successfully degraded nonadecane, heptadecane, hexadecane and octadecane, after 28 days of incubation. This same bacterium has also effectively degraded other components of hydrocarbons such as pristane, tetradecane and 2-methylnaphthalene [49, 50]. The degradation ability of this bacterium has been proven to be due to the production of a bio-surfactant. In another experiment, two biosurfactant-producing strains; *Pseudomonas* ML2 and *Acinetobacter haemolyticus* were inoculated into a hydrocarbon contaminated soil to monitor and study the biodegradation potentials. After two months incubation period, a drastic reduction in the hydrocarbon concentration (11–71%) and (39–71%) was observed by *Pseudomonas* ML2 and *Acinetobacter haemolyticus*, respectively. These results suggests the remarkable hydrocarbon degradation ability of cell free biosurfactant produced by bacteria. Several biosurfactants have been produced by various microbes which include: rhamnolipids (*P. aeruginosa*), liposan (*C. lipolytica*), surfaction (*B. subtilis*), emulsan (*A. calcoaceticus*), sophorolipids (*T. bombicola*), carbohydrate-protein-lipid (*Microbacterium sp*.) viscosin (*P. fluorescens*) and serrawettin (*S. marcescens*) [50–53].

3.1.2 Mechanism of action of bioremediation

Microbial-assisted bioremediation explores the potentials of naturally occurring hydrocarbon degrading microbes (oleophilic microbes) or plants in the detoxification/degradation/mineralization of hazardous substances to human health and the environment. These microbes can either be native to the contaminated area or could be introduced from a similar site into the contaminated soil, a process called bioaugmentation [54]. Bioremediation occurs most frequently by the action of microbial-mediated degradation. This process is often achieved by the action of consortia of organisms and for bioremediation to be effective, there must be complete mineralization of the hydrocarbon which occurs through a series of enzymes harboring catabolic genes to produce harmless products such as CO_2 and H_2O [55].

Biodegradation of petroleum hydrocarbons is a complex process that depends on the nature and on the amount of the hydrocarbon present. Petroleum hydrocarbons are divided into four broad categories: Saturates (branched, unbranched and cyclic alkanes), aromatics-ringed hydrocarbon molecules such as monocyclic aromatic hydrocarbons (MAHs) and polycyclic aromatic hydrocarbons (PAHs), resins (Polar oil-surface structures dissolved in saturates and aromatics) and asphaltenes (dark-brown amorphous solids colloidally dispersed in saturates and aromatics).

These various categories respond differently to biodegradation as a result of their chemical structures and molecular weight. For example, PAHs, asphaltenes and resins are considered highly recalcitrant because of their high molecular weight [56].

Microbial degradation is a major route and ultimate natural mechanism by which one can clean up petroleum hydrocarbon pollutants from soil environment [57]. Typically, an individual microorganism will biodegrade a limited number of hydrocarbons whereas a microbial consortium can biodegrade an impressive array of hydrocarbons collectively [58, 59]. Onuoha et al. [60] reported that Nigerian soil especially in the Niger Delta region, may harbor a significant population of hydrocarbon degraders as a result of the increased multifarious activities of the oil industry within the region. The result of the investigation revealed that an appreciable number of bacterial isolates showed different degrees of degradation in mineral salt medium using spent oil as sole source of carbon. In a similar study, Chikere and Ekwuabu [61] conducted an investigation in Bodo community, Ogoniland, Nigeria to characterize the active culturable indigenous hydrocarbon utilizing microbial population. A significant population of hydrocarbon utilizing bacteria and fungi corresponding to the long-term impact of crude oil in the study area was observed. The hydrocarbon degrading microbes have an inherent genetic capacity to assimilate hydrocarbons and/or its products [62]. The process is therefore regarded as a complex biological oxidation process involving mostly aerobic organisms which may be enhanced by supplementation with fixed nitrogen, phosphate and other rate-limiting nutrients. Microorganisms have enzyme systems that can degrade and utilize different hydrocarbons as source of carbon and energy [63, 64]. The driving force for petroleum biodegradation is the ability of microbes to utilize hydrocarbons, to satisfy cell growth and energy.

Biodegradation may occur spontaneously and the process is called natural attenuation. In most cases however, this might take a longer time and this could be as a result of inability of the natural circumstances of the contaminated site to favor the natural attenuation process [65]. Also, it may be due to inadequate number or diversity of microorganisms with specific enzyme system required to break down the contaminant and lack of favorable environmental conditions to support the process. Such situations can be improved by supplying one or more of the missing/inadequate microbes, developing oil eating bugs through genetic engineering/recombination, introducing rate-limiting nutrients or enhancing environmental factors to favor the active degraders. It was reported that extra nutrients were added to accelerate the breakdown of oil spill caused by the super tanker Exxon Valdez on the Alaskan shoreline [66]. Since numerous types of pollutants are to be encountered in a contaminated site, diverse species of microorganisms are likely to be required for effective mediation [67].

3.2 Microbial remediation

Microbial bioremediation strictly involves the use of microbes or their derivates (Enzymes, biomass) to degrade or transform xenobiotics for the detoxification of crude oil polluted environments. Microorganisms are ubiquitous, therefore pollutants in the different environments come in contact with these oleophilic microbes. Specifically, the hydrocarbon degrading microorganisms (bacteria, fungi, algae) are able to breakdown these pollutants because of their inherent genetic capabilities to mineralize these hydrocarbons through metabolic pathways. Microbial bioremediation technology in the long run promotes the growth of specific microflora or the microbial consortia, indigenous to the contaminated sites that are able to perform the desired activities. In the process, microorganisms use the contaminants as source of energy or nutrient. The microbial consortia can perform this role optimally by either adding terminal electron acceptor or promoting microbial growth by adding nutrients [27]. In oil contaminated sites as it relates to this review, oil spills can be broken down using multiple techniques which includes the microbes feeding on the crude oil or addition of fertilizers/nutrients to the contaminated site to accelerate the decomposition of crude oil by the microorganism present in the soil or by introducing hydrocarbon degrading bacteria from exogenous sources to augment the indigenous population. As regards to crude oil contaminated environment, bioremediation process exploits the catabolic ability of microorganism to feed on oil. Research frontiers globally have described various application of microorganisms in the bioremediation of oil pollution under controlled conditions, field scale and in different environmental conditions, with very encouraging results [13, 15, 55]. The natural existence of a large diversity of microbial species expands the variety of chemical pollutants that are degraded or detoxified [68].

So many microorganisms have been reported with hydrocarbon remediation potentials which are *Bacillus* spp. (degradation of hydrocarbons and phenoxy acetates) [15], *Pseudomonas* spp. (degradation of benzene, anthracene, and PCBs) [69, 70] also *Azotobacter* species (degradation of benzene and cycloparaffin) [70] and so many other microbes as previously discussed. White rot fungi have also been reported to have greater access to poor bio-available substrates, since they secrete extracellular enzymes involved in the oxidation of complex organic and inorganic matters [13]. For example, direct application and incubation of fungal laccase in hydrocarbon contaminated soils for 14 days led to the reduction of the PAHs such as benzo(a)pyrene and anthracene by about 80% [71, 72].

Microbial bioremediation technique has some advantages over other clean-up methods such as: public acceptance, a naturally occurring process, low cost technology, it can be done in situ and ex situ, instead of contaminants being transferred from one form to another or one medium to another, complete destruction of target organic pollutants is possible to produce non-toxic substance and it can lead to eco-restoration of the polluted medium [40, 68]. Some oleophilic microbes and their hydrocarbon specificity are presented in **Table 2.**

3.2.1 Factors that influence microbial bioremediation

There are a few factors that contribute to the success of microbial bioremediation, some of these factors may include the growth and survival of microbial populations, the ability of these organisms to come into contact with the substances that need to be degraded into less toxic compounds, cation exchange capacity, relevant nutrient availability, acidity (soil pH), aeration or oxygen (electron acceptor level), water solubility, temperature, enzyme activity, hydraulic properties, [31] water content, site condition, microbial communities, sufficiency of the numbers of microorganisms and the habitability of the microbial environment for the microbes to thrive [89]. Sometimes the environment might be too toxic for the microorganisms to survive, in this case, the microbes should be engineered to be able to survive the high toxicity. Also, bioremediation works best in soils that are relatively sandy because sandy soils allow mobility and greater likelihood of contact between the microbes and the contaminant [90]. Therefore, for any bioremediation process to be successful, the environmental factors that play major roles in the process must first be understood.

The process of bioremediation may not always result in the complete mineralization of organic compounds, some of the organic compounds are transformed naturally to other metabolites and the toxicity and persistence of these new metabolites are mostly unknown [91]. Compliance analysis requires examination of the contaminated site in the light of the governing regulation and the action plan.

Oleophilic micro-organisms	Type of microorganism	Hydrocarbon-specificity	Habitat	Reference
Pseudomonas spp.	Bacterium	Bezene, toluene, ethylbenzene, xylene, naphthalene, phenanthrene, kerosene and diesel	Soil, river and marine	[73, 74]
Alcanivorax spp	Bacterium	Alkanes	Soil, river and marine	[75]
Rhodococcus spp.	Bacterium	Anthracene, benzene, tolune, ethylbenzene, xylene and Benzo (a) pyrene	Soil, river and marine	[76]
Mycobacterium spp.	Bacterium	Benzo (a) pyrene and pyrene	Soil	[77]
Ralstonia spp.	Bacterium	Benzene, toluene, ethylbenzene and xylene	Soil, river and marine	[78]
Haemophilus spp.	Bacterium	Phenanthrene and pyrene	Soil, river and marine	[79]
Mesorhizobium spp.	Bacterium	Most PAH (not specific)	Soil	[80]
Bacillus spp.	Bacterium	Toluene and diesel	Soil	[74]
Thalassolituus oleivorans	Bacterium	Phenanthrene and pyrene	Marine	[79]
Alcaligenes spp.	Bacterium	Most PAH (not specific)	Soil	[80]
Proteus spp.	Bacterium	Xylene and diesel	Soil	[74]
Geobacter spp.	Bacterium	Anaerobic breakdown of benzene	Groundwater, deep soil and oceans sediments	[81]
Planococcus spp.	Bacterium	Light Arabian oil	Soil	[82]
Labrys spp.	Bacterium	PAH (not specific)	soil	[83]
Fundibacter spp.	Bacterium	Alkanes (not specific)	soil river and marine	[84]
Sphingobacterium spp.	Bacterium	Low molecular weight PAH	Soil/sludge	[85]
Tsukamurella spp.	Bacterium	Low molecular weight PAH	Soil/sludge	[85]
Corynebacterium spp.	Bacterium	Low molecular weight PAH	Soil/sludge	[85]
Rhodotorula glutinis var. dairenesis	Bacterium	A wide spectrum of petroleum hydrocarbon	Soil	[65]
Ochrobactrum spp.	Bacterium	PAH (not specific)	Soil	[86]
Fusarium spp.	Fungus	PAH (not specific)	Soil	[87]
Phanerochaete spp. *Chrysosporium* spp. *Cuuninghamella* spp. *Penicillium chrysogenum*	Fungus	PAHs (not specific)	Soil	[88]

Table 2.
Oleophilic microorganism and hydrocarbon specificity.

For a successful bioremediation, the site must first be examined and characterized and this is a very challenging and difficult aspect of a bioremediation efforts. Some factors that influence microbial degradation of hydrocarbons in the environment is present are presented in **Table 3.**

3.3 Plant bioremediation (phytoremediation)

This is one of the biotechnological approach/tools in which plants are used in the clean-up of contaminated environments. It is an emerging technology and it promises a cost friendly, less-intrusive and effective clean up and restoration of crude oil contaminated soils [65]. It can also simply be defined as a process of using plants and plant-associated microorganisms such as Arburscular Mycorrhizal fungi (AMF) or plant growth promoting rhizobacteria (PGPR) to clean up contaminated soils. It is an inexpensive, non-invasive alternative for other remediation methods such as the chemical/engineering-based methods [31]. Green plants are solar-driven, and are an effective filtering system endowed with fouling and degradative abilities [92]. It has been reported that salt marsh plants such as *Spartina alterniflora, Sagittaria lancifolia, Spartina patens* and *Juncus roemeriannus* are able to take up hydrocarbons from oil-contaminated sediment [93, 94]. Godheja et al. [95] reported that *Dioscorea* sp. have been reported to be able to metabolize petroleum hydrocarbons such as n-hexane and also Enzymes such as peroxidases and cytochrome P450 found in the plant *Dioscorea composita* was involved in the biotransformation of hydrocarbon.

In an experiment by Olusola and Anslem [96]. A plant (*Amaranthus hybridus*) was cultivated in a nursery and then transplanted into experimental pots containing crude oil contaminated soils. A white rot fungus (*Pleurotus pulmonarius*) and a mycorrhizal fungus (*Glomus mosseae*) were introduced into some of the different pots to study the ability and the degree of bioremediation of crude oil contaminated soils by these fungal species. The results showed that plants which were grown in the crude oil polluted pots without any of the fungal species died within two weeks while the pots with the soil samples which were inoculated with the fungus survived. The contaminated soil sample inoculated with *Glomus mosseae* showed the best result in terms of plant growth. They concluded that biological treatments are the best methods for cleaning or remediating contaminated soils. They also suggested that

Physical factors	Optimal conditions
Temperature	Affects the chemistry of the pollutants as well as physiology and diversity of pollutants. Optimal at 30-40°C in soil
Nutrient	Stimulates the growth of indigenous oleophilic microbes in the environment. C:N:P ratio – 100:10:1
pH	Soil pH affects availability of nutrients and it's important in the survival of microbes within a certain pH range. Optimal at pH 7 Acceptable range: 6–8
Moisture	Soil microorganisms require moisture for cell growth and function. Optimal moisture content for petroleum hydrocarbon degradation ranges between 45 and 85% of the water holding capacity.
Oxygen	Major degradation pathways for petroleum hydrocarbons involves oxygenates and molecular oxygen since most degradation process is aerobic

Table 3.
Factors that influence microbial degradation of petroleum hydrocarbon in the environment.

certain plants which have associations with microorganisms such as the AMF species *Glomus mosseae* could play roles in the clean-up of crude oil contaminated soils.

Plants and plant-associated microorganisms are both involved in phytoremediation process. The plants used must first be tolerant to the pollutants, encourage the growth of rhizospheric microorganisms and in turn these microorganisms can secrete oil degrading enzymes and thereby generate energy in a process called rhizodegradation. However, there is a major setback with this process in that plants tend to compete with the hydrocarbon degrading microorganisms for the available nutrients like fixed nitrogen and phosphorus.

3.3.1 Mechanisms of action of phytoremediation

Phytoremediation offers potential for restoring large areas of contaminated environments requires certain mechanisms for a successful remediation process. Plants are able to remove pollutants through processes such as biodegradation, phytovolatilization, accumulation, and metabolic transformation. Several factors determine the most effective phytoremediation mechanism to adopt, such as the bioavailability of the contaminant, type of contaminant, soil properties and other environmental factors that support plant growth and activities. There are several routes through which plants decontaminate polluted sites, however, the primary channel for plant uptake of contaminants is through the root systems (rhizosphere) which harbors the essential components required for decontaminating toxic substances. The rhizosphere of plants has a large surface area responsible for the absorption and accumulation of essential nutrients and water required for growth. A large diversity of microorganisms are usually found in this region because of the exudates and enzymes released which stimulates the activities of microorganisms capable of degrading hydrocarbons present in the soil, direct biochemical transformation of petroleum hydrocarbons, and have also shown resistance to crude oil toxicity [97]. Rhizospheric interactions between host plants and the microorganisms that are resident in the rhizosphere are critical to the phytoremediation process. Host plants enrich the rhizosphere by releasing root exudates that help in recruiting the beneficial pollutant degrading bacteria and other microorganisms to the rhizosphere. In a report, a plant growth promoting rhizobacteria *Pseudomonas putida KT2440* was recruited due to the production of 2,4-dihydroxy-7-methoxy-1,4-bezoxazin-3-one, a root exudate produced in Maize seedlings [98].

There are several other phytoremediation mechanisms which include; phytoextraction, phytostabilization, phytofiltration, phytodegradation, phytovolatilization rhizodegradation and phytostimulation (**Figure 1**). *Phytoextraction/phytoaccumulation* is a process of absorption or translocation of contaminants from the roots to other parts of the plants. Rhizofiltration is a process which involves the roots removing contaminants from water bodies, thus causing the water to be filtered. This mechanism is closely related to phytoextraction but it strictly applies to the aquatic environment. Phytostabilization involves binding the contaminants to the roots of plants which leads to the immobilization of the contaminants and thus reducing leaching of the contaminants from soil [98]. Phytodegradation involves the secretion of exudates by the roots to break down contaminants which are then removed via transpiration and uptake. Phytostimulation involves the enhancement of the microbial activity in the rhizosphere to facilitate the breakdown of organic contaminants. Phytovolatilization is a process that involves the removal of contaminants from the soil by volatilizing them into thin air [99]. These various methods have proven to be effective for petroleum hydrocarbon degradation.

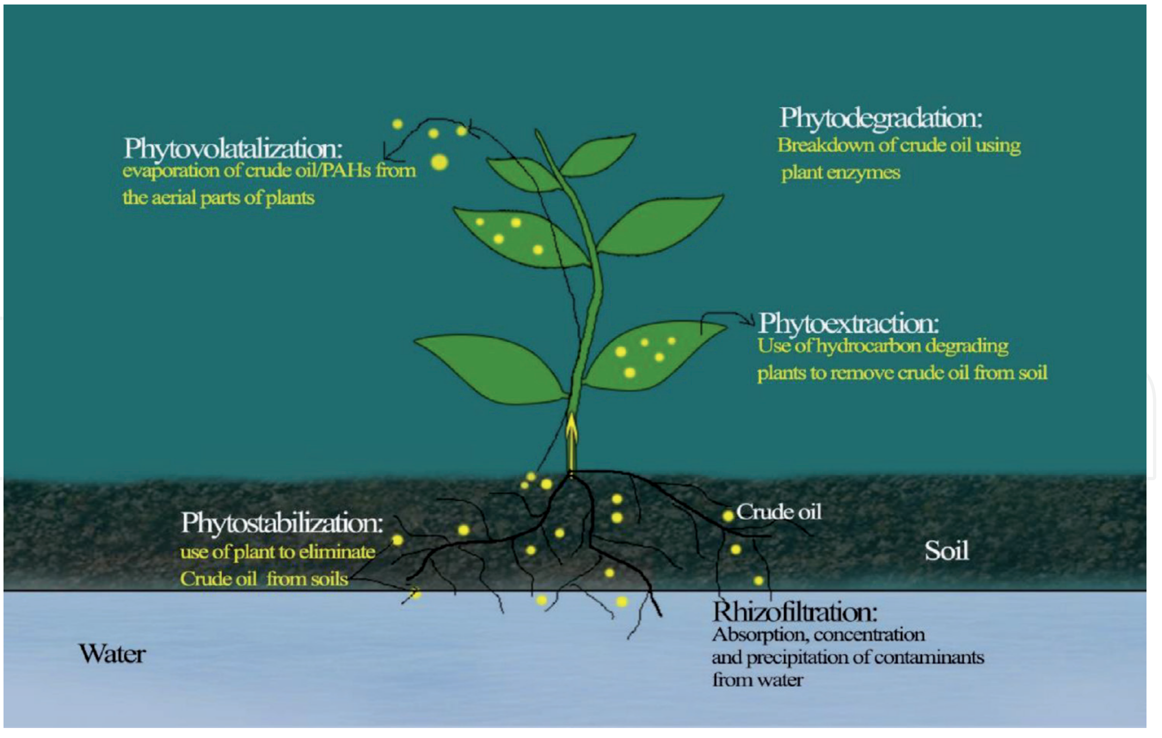

Figure 1.
Mechanism of phytoremediation of crude oil.

3.4 Genetically modified microorganism for enhanced eco-recovery

Generally speaking, microbial degradation of xenobiotics involves the utilization of microbes with specific enzyme systems responsible for the degradation, mineralization, transformation or detoxification of pollutants [100]. Nevertheless, under certain growth conditions, composition, type and concentration of the pollutant, effective degradation is not expected even with the availability of microbes with degradation potentials. Compounds like Polychlorinated biphenyls (PCBs), synthetic group of chlorinated aromatic hydrocarbons and other Organic compounds, due to their complex organic structure, is posing persistent and deleterious threats to the ecology and human health even for decades [101–107]. Therefore, it has become imperative to design and develop alternative hydrocarbon degradation arrangement with specific degradation genes to the available pollutants in the environment by cultivating microbes with engineered catalytic capabilities [108].

Genetically Engineered Microorganisms (GEMs) can be obtained by recombinant DNA technology/genetic engineering of microbes or by natural exchange of genes by bacteria in the environment through horizontal gene transfer of plasmid-borne genes. The application of GEMs in bioremediation of xenobiotics have shown great potentials in soil [103], groundwater [102] and other environmental components exhibiting improved mineralization abilities over a broad-range of contaminants.

The use of GEMs represent a research frontier with wide application which extends to phytoremediation. Jain and Bajpai [108] reported a number of applications available in enhancing the degradative performance of oleophilic microbes using genetic engineering approaches. A very significant example is the genetic modification of rate-limiting steps in the metabolic pathway of hydrocarbon degrading microbes to yield increase in mineralization rates or the development of completely new metabolic pathways incorporated into the bacterial strains for the degradation of highly persistent compounds.

The first GEM, *Pseudomonas fluorescence* HK44GEM was designed to perform diverse functions in petroleum hydrocarbon degradation. The wild type, *Pseudomonas fluorescence* strain was cultivated from a PAH contaminated soil. Naphthalene catabolic compound (Vector PUTK21), a transposon-based bioluminescence producing lux gene fused with promoter naphthalene catabolic gene were introduced into the *P. fluorescence* to form *P. fluorescence* HK44GEM. Upon trial in the presence of naphthalene or its intermediate (Salicylate) enhanced catabolic gene expression, naphthalene degradation and concomitant bioluminescent response was observed. The GEM was capable of sensing and responding to environmental pollutants through an early detectable signal such as bioluminescence. The bioluminescence signaling in strain HK44GEM also served as a reporter for naphthalene bioavailability and biodegradation.

Additionally, since oil is a mixture of various hydrocarbons (n-alkanes, aromatic hydrocarbons, polycyclic aromatic hydrocarbons), the construction of engineered bacteria capable of degrading various petroleum hydrocarbons by genetic engineering technology is a development direction to control crude oil pollution. The degradation of some petroleum components by microorganisms is controlled by an extrachromosomal plasmid; therefore, superbugs (product of genetic engineering: oil eating bug) can be constructed by introducing plasmids with capabilities for degrading different components in a single cell.

A recombinant *Acinetobacter baumannii* S30 pJES was constructed by inserting the lux gene into the chromosome of the *A. baumannii* S30, a strain with the biodegradation efficiency for total petroleum hydrocarbon (TPH) of crude oil. Thus, the persistence of strain *A. Baumanni* S30 PJES was observed and confirmed at the bioremediation site after the genetic engineering process site [109]. Also, a recombinant strain M145-AH constructed by overexpressing alkane monooxygenase (encoded by alkB gene) in a non-alkane-degrading actinomycete *Streptomyces coelicolor* M145 wasto exhibit a high ability observed to degrade n-hexadecane [110].

Genetically modified microorganisms such as bacteria including *E. coli* and *Pseudomonas*, fungi including *Aspergilus niger* and *Rhizopus arrhizus* and also algae, e.g., *Chlorella vulgaris* and *Anabaena variabilis* and others microbes, have been engaged in degradation of various compounds such as toluene, oil spills, naphthalene, camphor, hexane, octane, xylene, halobenzoates and others. Engineered microbes are more potent than the natural strains when it comes to degradation due to their higher degradative capacities. Advantageously, this engineered microorganism can quickly adopt pollutants as their substrates [111–114].

4. Future prospects

Microbe-assisted contaminant reduction and in-depth analysis of the organisms' metabolisms have over time accelerated the overall bioremediation process. However, in the next decade, molecular manipulations and the decryption of the cellular mechanisms using an integrated OMIC tool approach will play major roles in bioremediation processes [115].

Recently, a key area of modern-day scientific advancement in the removal of pollutants from the environment (either in soil or groundwater) is the nanoparticles empowered remediation. Green nanoremediation as a nature-based technology offers numerous promises for the cleanup and restoration of polluted soils such as crude oil polluted soils with reference to the efficiency of the process, energy consumed and the global need for eco-friendly processes [116]. Wang et al. [117] reported the use of silica nanoparticles capped with lipid bilayers of *Pseuodomonas aeruginosa* as method of cleaning up of PAH (benzo[a]pyrene) from contaminated soil surface.

Some of the all-round benefits of the use of green nanotechnology as a biotechnological tool for remediation of crude oil polluted sites may include the rapid removal of pollutants, reduced usage of hazardous substances and the cost effectiveness. Nanobioremediation might contribute immensely to the sustainability of the environment because of these benefits when compared to other methods of remediation. The copulation of biological entities with nanomaterials have furthermore demonstrated enhanced effectiveness in the degradation of contaminants in soil and water. This can be seen as a future possibility in facing environmental challenges. Dave and Das [118] reported that nanoparticles can potentially bind with xenobiotic compounds and can either transform them into less harmful byproducts or completely degrade them, this process can help in the clean-up of contaminated environment. The requirement for any ideal bioremediation process relies on the use of an environmentally friendly and efficient approach. These above-described technologies are complete for the effective bioremediation process. Also, as part of Nanotechnological tools in bioremediation, nanobiosurfactants provides unique properties which makes them potentially strong candidates for ecofriendly nanobioremediation in the future [119].

Some inorganic sensors have also been developed and applied in nanotechnology [120] to trace and identify contaminants/pollutants in the environment which will inform the most suitable/appropriate biotechnological tool to be applied for clean-up process. In another study, it was reported that the use of oxygen-sensitive proteins to develop oxygen biosensors is an emerging field which can be adopted for the preparation of nanomaterials that are able to respond to oxygen levels and other specific components of pollutants [121]. Ryu et al. [122] extensively reviewed the field of Transmembrane proteins which were incorporated into membranes coupled to several trandusctors and observed that this approach can be successfully applied in pesticide detection, monitoring of gases, microarray etc. Based on this, [123] recommended that in the future, hydrocarbon catabolic enzymes may also be incorporated to monitor more complex pollutants such as Polycyclic aromatic hydrocarbons (PAHs). These concepts, explored with proteins will open a wide area of sensing and detoxification opportunities in bioremediation. Techniques such as biofilm formation and whole-cell immobilization for the removal and recovery of soils containing pollutants such as heavy metals and PAHs have also gained attention [124].

5. Conclusion

The various biotechnological tools in ecorestoration of crude oil polluted environment outlined in this review has been confirmed to be eco-friendly and effective in the mineralization of the pollutants. Biosurfactant-producing microbes contribute significantly to enhancing microbial bioremediation by increasing bioavailability. Microorganisms produce a wide range of surfactants with hydrocarbon specificity. Microbial bioremediation and phytoremediation have both yielded positive results in environmental studies under favorable conditions and growth conditions, respectively. The genetic engineered microbes in bioremediation favor the degradation of recalcitrant hydrocarbons and increase the rate of degradation. Although this method is still under investigation based on environmental and ecological risk. This review has highlighted known eco-friendly approaches of bioremediation of polluted sites using several biotechnological tools.

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