Advanced Journal of Environmental Sciences
Volume.16, Number 8; August, 2025;
ISSN: 2837-3928| Impact Factor: 9.38
https://zapjournals.com/Journals/index.php/ajes
Published By: Zendo Academic Publishing

ECOTOXICOLOGY AND BIOREMEDIATION OF HYDROCARBON-POLLUTED SOILS: A NARRATIVE REVIEW

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Article Info
Keywords: Petroleum, contamination, Bioremediation, Genetic engineering, Nanotechnology, Artificial intelligence
DOI
10.5281/zenodo.16994472

Abstract
The threat of increasing pollution on the health of living organisms and their local ecosystem by contaminants of petroleum origin in the environment is a worldwide concern. The development of efficient, economical, and improved techniques aimed at mitigating any known or further catastrophic backwash effects that the accumulation of petroleum contamination may give rise to has prompted wide research. This review takes a significant look into the ecotoxicological nature of petroleum contamination and pollution in the environment and how the inculcation of genetic engineering—in producing better and more resistant species of degraders, nanotechnology—in improving bioprocesses through environment and bioprocess modifications, and artificial intelligence—through bioinformatics, etc.—have so far effectively enhanced biological means of remediating petroleum hydrocarbon (PHC) contaminated sites. Conclusively, the infusion of advanced engineering techniques (i.e., artificial intelligence, genetic engineering, and nanotechnology) in achieving biological remediation has greatly improved remediation processes, producing credible, more efficient results and a reliable established database for forecasted cases of petroleum pollution, compared to contemporary methods of bioremediation.

Introduction

On average, contaminated soils across the globe have total petroleum hydrocarbon (TPH) concentrations in an alarming range of 1.17–236.7 g per kilogram of soil [1], of which the oil in the soil can penetrate to a depth of more than 10–30 cm [2], eventually degrading environmental properties and affecting water bodies, land, and vegetation of the crude oil-impacted areas (Figures 1 and 2).

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Figure 1: Crude Oil Polluted Land **Figure 2: Crude Oil Polluted Water Body**

Amro et al. [3] reported that soil surfaces on oil spill sites are characterized by high adsorption, resulting in very low infiltration depth and fluid migration, which is advantageous for preventing contamination from reaching the water table. According to The Oil World, Chennai, India [4], published in 2011, the world consumption of petroleum stood at approximately 100 million barrels per day, and this is steadily increasing due to the rising dependence on petroleum and its by-products. Petroleum contamination is restricted to terrestrial and aquatic environments. The activities of the upstream and midstream sectors of the petroleum industry continue to promote the degeneration of the local environment, such as oil spillage from production and effluent disposal from production and refining processes, coupled with the massive production of methane and CO_2 from gas flaring. Oil-based mud is used as an anti-corrosive agent during the petroleum drilling process because of its excellent lubricating features and stability at high temperatures. However, the large amount of oil-based mud waste generated from this process is often disposed of into the environment untreated, leading to potentially grievous impacts on living organisms (animals and humans) and the environment [5, 6]. If the earth must continue to sustain humanity and the natural ecological system at large, it must be rid of pollutants such as those of petroleum origin.

The Ecotoxicological Impact of PHC Pollution

Petroleum emancipation into the environment has been a major contributor to the health challenges of living organisms, massive pollution, and consequential destruction of the natural ecosystems in which they are found. The fatality of living organisms resulting from the toxicity of the polluted environment has propelled by increased anthropogenic activities with large dependence on its products. Over the years, the world has recorded numerous instances where petroleum contamination has resulted in environmental destruction and even to a fatal extent. One such event is the tragic Exxon Valdez oil spill of 1989, which informed the United States and the world at large of the lack of preparedness of the petroleum industry and country in situations such as petroleum contamination [7].

Relating this subject to one of Africa's and the world's sixth-largest producers, Nigeria, coupled with her seemingly ever-growing dependence on refined products, is all too familiar with the story of pollution caused by crude oil and its by-products [8]. Crude oil constitutes a major source of pollution in the environment (Figure 3). The high rate of dependence on petroleum, its products, and by-products for automobiles, generators, machines, and in great quantities for operation and running of factories, industries, companies, and various establishments in their day-to-day operations.

Figure 3: (a) A farmer in the Niger Delta region of Nigeria smeared with crude oil (b) Young villagers trying to get clean water from a river contaminated with crude oil in Ogoni land, Niger Delta region of Nigeria (c) Land pits polluted by crude oil in the Bodo community, Niger Delta, Nigeria (d) Periwinkles smeared in crude oil in the Niger Delta region of Nigeria (Macaulay et al., 2018).

The constant power failure in all parts of Nigeria has not helped the status quo and is therefore promotive of petroleum product making its consequential downside continue unabated since the masses can turn toward no other available means for energy. The daily need for environmental remediation has arisen since urbanization has increased exposure to petroleum pollution through inefficiencies and faults in the mode of exploration and exploitation, inefficient management and control of effluents from production and refining, transportation, and disposal of petroleum by-products. Oil pollution has caused great harm and destabilized the natural ecological system. In humans, oil pollutants have been associated with poor mental, physical, and physiological health and caused genetic, immune, and endocrine disruptions [8]. These pollutants are linked to reproductive failures, physiological stress, and increased mortality in aquatic communities [9]. In addition, there have been reports of drastic reductions in seed germination, increased soil microbial community intoxication, and reduction in soil fertility where these oil pollution have occurred on land [9]. Thus, the devastating effects of environmental oil pollution necessitate effective remediation methods. Although there is a growing concern about the ecotoxic effects of petroleum pollution on living organisms in affected areas, the severity of its toxicity on affected microbial communities is often left unmarked. This pollution has an attendant effect on both plants and animals, including humans, which depend on the soil ecosystem for their survival. Soil contamination with hydrocarbons causes extensive damage to local ecosystems because the accumulation of pollutants in animal and plant tissues may cause progeny death or mutation [10]. In Nigeria, an endless number of polluted sites exist, mostly in the Niger Delta region, as a result of petroleum production activity. Recently, this problem has motivated researchers to recover these polluted sites [11]. Oil pollution in soils of the terrestrial environment has become a major threat to the ecosystem and humans through the transfer of toxic materials, including polycyclic aromatic hydrocarbons (PAHs), into the food chain. Specifically, crude oil components (such as PAHs) have mutagenic, teratogenic, carcinogenic, and even immunosuppressive properties [12]. Humans are not excluded because by food chain transfer of such recalcitrant components, they become indirect victims. However, there is a need to promote cost-effective methods and green technology for proper remediation for an assured sustainable future.

Nature and Composition of PHs

The widespread effect of hydrocarbon contamination has been of great trouble to all life forms in the affected environment. Petroleum is a complex mixture of aromatic, aliphatic, and heterocyclic compounds. Soil naturally contains heavy metals in different concentrations. However, due to human activities, such as oil refining and disposal and use of pesticides, their concentration is on the rise. Petroleum contamination is common because of its extensive use and management, related disposal processes, sabotage, and accidental spills. Petroleum is a complex mixture of a large range of high and low molecular weight hydrocarbons. The complexity is made up of saturated and branched alkanes, alkenes, homotrophic and heterotrophic naphthenes; aromatics consisting of heteroatoms, such as heavy metal complexes and nitrogen, sulfur, and oxygen; hydrocarbons made up of various functional groups, such as ethers and carboxylic acids; and larger aromatics, such as asphaltenes, resins, and naptheno-aromatics [13] (Figures 4 and 5).

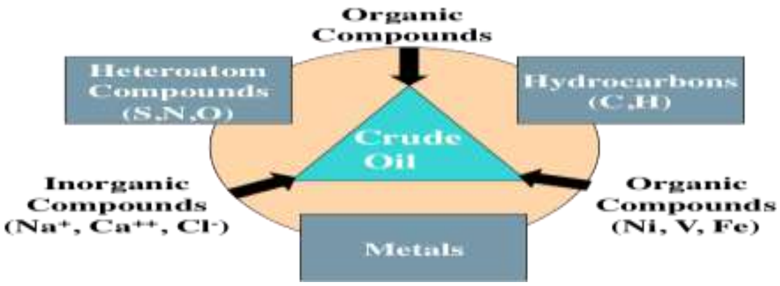

Figure 4: Crude Oil Complexity (COC)

Through cracking and refining, petroleum gives birth to various compositions, such as lube oil, waxes, diesel, kerosene, light oil, asphaltenes, naphtha, and many more, with different hydrocarbon compositions categorized into light and heavy petroleum ends. Light ends consist of a lower percentage of aromatic compounds and saturated and unsaturated hydrocarbons with lower molecular weights. Heavier ends consist of higher molecular weight saturated and unsaturated hydrocarbons and organometallic compounds [14].

Hydrogen Family	Most Important Hydrocarbons	Chemical Characteristics	Comments
Hydrocarbons			
Naphthenes	Cyclopentane, methyl cyclopentane, dimethylcyclopentane cyclohexane, and 1,2 dimethylcyclohexane	5–6 carbon atoms in the ring	➢ R_n is the number of naphthenic rings. ➢ Normal crude oil contains approximately 50% naphthenes by weight.
Paraffins **(Alkanes)**	Methane, ethane, propane, butane, pentane, and hexane	Straight carbon chain	➢ The boiling point rises as the number of carbon atoms rises. ➢ Paraffin turn into wax, as carbon numbers ranges 25–40.
Iso-paraffins **(Iso-alkanes)**	Isobutane, isopentane, neopentane, isooctane	Branched carbon chain	➢ The number of isomers rises with increasing the carbon atoms number.
Olefins **(Alkenes)**	Ethylene	One pair of carbon atoms	➢ Olefins do not exist in crude oil; however, they are formed during oil processing. ➢ Olefins are undesirable in the end product due to high reactivity. ➢ Olefins with low molecular weight have better antiknock characteristics.
Aromatics	Benzene, toluene, xylene, ethyl benzene, cumene, and naphthalene	Six carbon atoms in a ring, with three around the linkage	➢ Aromatics are not necessary in kerosene and lubricating oil. ➢ Because benzene is carcinogenic, it is an undesirable component of gasoline.
Non-hydrocarbons			
Oxygen compounds	Naphthenic acids and Phenols	NM	➢ The content of these compounds is 2%. These acids not only cause corrosion problems at various stages of processing, but also cause pollution problems.
Sulphur compounds	Hydrogen sulphide and mercaptans	NM	➢ These are undesirable due to their foul odor (0.5% to 7%).
Nitrogen compounds	Quinoline, pyridine, pyrrole, indole, and carbazole	NM	➢ Upon exposure to sunlight, nitrogenous compounds in kerosene and gasoline degrade the color of the product. ➢ The effect of causing gum formation is normally less than 0.2%.

NM = Not mentioned.

Figure 5: Petroleum composition (hydrocarbons and non-hydrocarbons)

In 2018, Nzila [15] in 2018 pointed out that the molecular weight of hydrocarbon pollutants greatly affects their ability to be broken down biologically and otherwise. Bioremediation of PAH-contaminated soils in the presence of heavy metals is often difficult due to the ability of toxic metals to inhibit PAH degradation by microbes [16]. Because of its complex nature, extensive dependence, movement, and disposal problems, pollution has become progressively alarming. Its complex nature accounts for some of the problems encountered because of its exposure to the natural environment in the use, conveyance, and disposal management of products and by-products.

Conventional Bioremediation

To stem the effect of these pollutants, several techniques have been developed to restore the ecosystem to its original course, including chemical degradation/remediation, physical degradation/remediation, thermal degradation/remediation, biodegradation/bioremediation/biological degradation, and a combination of the degradation methods. Some of these methods are advantageous in ridding the environment of pollutants but disadvantageous in other ways, such as destroying soil biota. This is not the case for biological means of remediation. Bioremediation is a sustainable approach that can destroy pollutants or convert harmful contaminants into harmless substances [17] while preserving the soil structure and ecosystem, with the advantage of treating polluted soils in situ [18]. Therefore, there is an ever-increasing call for research into the use of microbes (preferably those domiciled in polluted environments) for the cleaning up of polluted environments. This method has proven to be very successful in the rapid removal, eradication, detoxification, and cleaning up of petroleum wastes and PAHs. Many methods have been discovered and applied in the recovery of contaminated environs, of which bioremediation has been of primary study. The development and improvement of different technologies (such as biotechnology and nanotechnology) for the remediation of areas contaminated by hydrocarbons are currently a field of active basic and applied research.

Bioremediation is an eco-friendly and economical approach to reclaiming polluted sites. In general, sites polluted by petroleum, heavy metals, pesticides, and all sorts of degradable environmental intoxicants have been proven to be redeemable via biological means of remediation, with the assistance of suitable living organisms. Bioremediation enhances the disappearance rate of crude oil hydrocarbons in soil [19]. Conventional bioremediation involves the removal, degradation, immobilization, and detoxification of diverse chemical wastes and hazardous physical materials from the environment through the sole activity of living organisms. Bioremediation converts toxic matter into less toxic or non-toxic matter. Bioremediation is facilitated by three primary components: microorganisms, nature of the environment, and nutrients. The presence of contaminants in soil or water serves the purpose of providing nutrients for microbial growth, with petroleum being the carbon source in this case. Microbes obtain energy from the redox reaction, which results in electron transfer, leading to the breakdown of pollutants into non-toxic products. The desired bioremediation efficiency can only be achieved when microorganisms are capable of enzymatically attacking the contaminants and further converting them into non-toxic products. For this to happen, optimum environmental conditions and levels of essential nutrients and chemicals are needed to be supplied for microorganisms to detoxify the pollutants [20]. Soil pollution by petroleum products is a widespread problem today. In general, petroleum pollution poses various negative impacts on soil and elevated levels of production and emancipation into the environment unbalances the ecosystem. Of all the known methods applicable in the remediation of PHC contaminant sites, two or more can be applied synergistically in the remediation and recovery of PHC. Over time, bioremediation of PHC sites has been shown to be the best and most effective means of recovering PHC sites [21] due to its ecological, cost-effective, and publicly acceptable benefits. Bioremediation is a technology that employs living organisms (bacteria and fungi) in soil detoxification and removal of contaminants, such as petroleum and its by-products. Although microbial bioremediation is eco-friendly and cost-effective, it is quite time consuming and requires technological advancements for increased efficiency of the process [22]. This reliable approach is the focus of remediation researchers and has gained wide popularity in the environmental research space. Bioremediation is viewed as a technique to accelerate the natural biodegradation process in a cost-effective and environmentally friendly manner. However, the contaminant concentration and composition, temperature, soil pH, oxygen condition, and salinity, among others, highly affect the success of petroleum-contaminated soil bioremediation [23].

In many biological or biochemical processes, such as bioremediation, the microbial characteristics (such as population and morphology) are of notable importance to the effectiveness and success of the degradation process and could be considered rate limiting if not potentially maximized. According to Darwin's Theory of Evolution which proposed that 'species can change overtime; that new species come from pre-existing species, and all these species share a common ancestor…' and Darwin's Survival Theory which proposed that 'organisms' best adjusted to their environment are the most successful in surviving and reproducing…'. Hence, when an environment is contaminated by a toxic pollutant, the ecosystem is thrown off its natural balance and would be fated for the destruction of soil structure and biota (organisms and their progeny). Their toxic impact could be in vivo and ex vivo (in vitro) because some microbes exist as unicellular organisms and cytotoxic matter can permeate their cells to a certain degree. However, according to **Darwin's theories**, organisms that are capable of evolving (i.e., undergo genetic metamorphosis to adapt to the environment) are more capable of surviving unpleasant environmental conditions such as low aeration, high toxicity, and high pH than those that are incapable and consequently suffer and even perish. This is one reason why the native/indigenous microbe in the polluted environment is more promising in remediating the polluted soil because the introduction of foreign microbe(s), for example clinically sourced microbes, which have not existed in such an environment may be ineffective as they may die off overtime or take time to adjust and adapt to the new environment before remediation can begin. Such polluted environments tend to have a low population of degraders (i.e., hydrocarbonoclastic bacteria) due to the high mortality of organisms (especially microbial) and hence experience a very slow remediation process – microbial population becomes a rate limiting factor. This bioremediation is termed natural attenuation; the microbial community depends on its natural strength and capacity. However, indigenous bacteria form extensive aggregates with each species performing specific functions. While some bacteria sensitive to petroleum hydrocarbons are inhibited upon exposure to petroleum and its contaminated environs, those efficient degraders of petroleum hydrocarbons, as well as those resistant to cytotoxic metabolites, will flourish in such contaminated environs. The strength of bioremediation is to a large extent dependent on the remediating community size. However, in natural soil attenuation, the sole reliance on the strength of indigenous microbial communities for the clean-up of affected areas will make the process span a longer period of time; hence, the development of interventionary measures in accelerating and sustaining degradation is necessary. To speed up the remediation process, a practice termed bio-augmentation must be adopted. Bio-augmentation in a bioremediation process is a technique that aims at multiplying or amplifying the population of degrading organisms in contaminated/polluted environs to speed up the remediation process. This process can be performed in situ (as field remediation is ongoing) or ex situ (by incubating microbes in the laboratory using nutrient agar). In situ remediation techniques include land tillage, microorganism addition, bio-culture, and bio-ventilation, whereas ex situ remediation technologies include prefabricated beds and bioreactors [24, 25]. The process of augmenting can be time consuming. Bio-augmentation has its own downsides; often, inoculated species vie with the native-indigenous species over essential nutrients, thereby disrupting the local ecosystem. Furthermore, the success of a bio-augmentation process is highly dependent on the knowledge of oil composition and indigenous species, including their degradation capabilities and what species can have a successful synergistic relationship if inoculants are introduced. Bio-augmentation has been beneficial for enhancing bioremediation because these microorganisms can exist in population ranges from as low as 0.003% to near 100% of the microbial community, although hydrocarbonoclastic species are ubiquitous [26]. Researchers seek to overcome low indigenous microbial concentrations through bio-augmentation (i.e., the addition of microbes with previous exposure to hydrocarbons), biostimulation (i.e., the addition of growth-limiting nutrients), or a combination of the two [26]. In other ways, the augmentation process is accompanied by a simultaneous stimulation process, i.e., making use of bio-stimulant(s) in a process called bio-stimulation. Bio-stimulants are additives other than the contaminant whose duty is to arouse the activity of the degraders in the remediation process and are usually counteractive to those of the contaminants. Their characteristics promote microbial growth and survival by modifying the environment to stimulate bioremediation of existing bacteria. Biostimulation of oil spills has increased the growth of oil-degrading species [27, 28, 29, 30, 31]. The manipulation of environmental parameters is necessary to allow simultaneous microbial growth and remediation to occur at a faster rate [32]. This is achievable through the introduction of various forms of rate-limiting nutrients and electron acceptors, such as phosphorus (P), nitrogen (N), oxygen (O), or even carbon (C). The oleophilic fertilizer Inipol EAP 22 (composed of 7.4% nitrogen and 0.7% phosphorus) and custom-blend slow-release fertilizer (composed of 28% nitrogen and 3.5% phosphorus) were used to treat shorelines following the 1989 Exxon Valdez oil spill of 1989 [26]. Over a period of 109 days, the initially treated sites were found to have 44% degradation of total polynuclear aromatic hydrocarbons and 63% degradation of the total detectable HCs. Dissimilarly, the control sites untreated with fertilizer showed no detectable degradation over a 70-day period; in other words, the degradation was latent. However, on adding fertilizer to control sites on the 72nd day, degradation was observed, demonstrating the efficacy of biostimulation via fertilizer application [28]. Biostimulation can change the microbial consortia, and its effectiveness is site specific depending on several factors, including oil composition, indigenous species, and local environmental conditions [29]. Although water is a necessity for microbial growth, it could be a rate limiter, capable of reducing the potency of a biostimulation process as supplementary nutrients can be diluted in this case (beyond certain concentrations). The addition of water-insoluble UA offers an alternative solution in such cases [33]. The low solubility of uric acid in water makes it usable as a source of nitrogen at the oil-water interface—a less likely to be diluted supplement of nitrogen. Nitrogen and Phosphorus are required by virtually all microorganisms and can enhance the replication of both desired and undesired microbes in a biostimulation process. This will lead to competition for hydrocarbonoclastic species. Targeting oil droplets for nutrient release has proven to be a helpful feat for oil-degraders [30]. Biostimulation involves the introduction/addition of various forms of rate-promoting nutrients and electron acceptors (such as phosphorus, nitrogen, oxygen, and/or carbon), which may be found in degradable additives (**biostimulants**) containing the required nutrients. Biostimulation promotes the activity of the degraders in the remediation process by having some characteristics that are counter relative to those of the contaminants. Bioaugmentation is a process/technique that aims to multiply or amplify the number of degrading microbes in the contaminated environment by introducing foreign microbes to accelerate the remediation process. This can be performed in situ or ex situ. The use of good biostimulants in a bioremediation process can promote bioaugmentation. For example, in recent years, empirical studies and research have shown that agricultural wastes, such as animal dungs, which are potential hosts to microflora, have been used as **biostimulants** and **bioaugmentors**. Their characteristics support and promote microbial growth and survival by modifying the environment to stimulate bioremediation of existing bacteria.

Biochemical Remediation Process Explained

Petroleum/crude oil exists as a viscous mixture of hydrocarbons with comparable low concentrations of impurities, such as sulfur and nitrogen. Owing to its high viscosity, insolubility in water, and impermeability in air, its presence on the soil surface forms a blanket-like covering on the surface of the soil, and in the soil, it tends to clog the pore spaces owing to a resultant decrease in aeration (oxygen availability) and increased ecotoxicity. The clogging of soil pores and decreased influx of air coupled with increased toxicity (such as decreased pH and increased metal concentration) is the reason for the rising mortality. The presence of burrowers amongst the degraders in the soil and subsequent periodic soil tillage is an added advantage in soil remediation because air (oxygen) availability is required for redox reaction to take place and bioremediation is a redox reaction process.

$$\mathbf{C_xH_y.R_zCH + \left(x + \frac{y}{4}\right)O_2 + Bioagent(s) + Energy \rightarrow xCO_2 + \frac{y}{2}H_2O + R_zCH}$$

…where -R_zCH could be any of the by-products from the remediation process.

The use of an electron acceptor, such as oxygen, to initiate the oxidation of a reduced pollutant, such as hydrocarbons, could be involved. To tackle this challenge, most biodegraders secrete active compounds that are produced at the microbial cell surface or are excreted, which then attack contaminants to reduce surface and interfacial tension. Persistent organic pollutants of hydrocarbons are be degraded by the catabolic action of various extracellular and intracellular active compounds (enzymes) found in different degrading microorganisms [34]. These active compounds/bio-agents are called bio-surfactants, and their secretors are called bio-emulsifiers. They cause emulsification, thereby making it easier to breakdown the contaminant and reduce poor contact between oil and microbes (because hydrocarbons are highly hydrophilic). In 1970, scientists introduced oil herders as an oil remediation tool, capable of facilitating in situ combustion for physical oil removal [33]. Oil herders comprise amphiphiles, i.e., particles/molecules that have affinity for air-water interface. When they are sprayed on water near the oil spill, they lower the surface tension at the interface of air and water by means of adsorption, giving a resultant negative spreading coefficient and confining the oil slick [35]

Surfactants are a type of macromolecule with both hydrophilic and hydrophobic groups and are of microbial origin, some of which are enzymatic in nature, offering several advantages over synthesized surfactants. They offer advantages such as low toxicity, high biodegradability, and high activity even at extreme pH and salinity. The process of emulsification by surfactants helps the remediation process to become easier and increases bioavailability as the microbial community makes more available and accessible the broken down pollutants. Bioavailability is a term that defines the extent to which the contaminants may be available for biological conversion; a factor to be considered in the remediation of contaminated sites, greatly encouraged by the action of bio-surfactants/bio-emulsifier production (a property of bacteria shown in Figure 6) which increases bioavailability of insoluble hydrocarbons [36, 37, 38].

Figure 6: Biosurfactant-assisted bioremediation

Biosurfactant-producing Rhodococcus in biopreparation are being used in petroleum-polluted environments. This is feasible because biosurfactants are of organic origin (i.e., green), ecofriendly, and preferred over commercially synthesized enzymes. Surfactants increase the solubility of hydrophobic components and decrease the surface tension as the surface area increases, breaking down insoluble components into smaller droplets at the water-oil interface, making them available for microbial degradation. This is necessary for possible degradation, especially in aquatic environments. Biosurfactants secreted by microorganisms can be absorbed onto cell surfaces, increasing the hydrophobicity of the cells [39]. Such surface engineering approaches are promising techniques for enhancing bioremediation by increasing the rate of cell adherence to the oil–water interface, thereby enhancing degradation abilities [39, 40]. In addition to being ecofriendly, organic surfactants are biodegradable and therefore cannot contribute to environmental pollution. Surfactants play an important role in biodegradation success. Surfactants reduce the size of oil droplets, increase the surface area per unit volume, and consequently increase the bioavailability of the oil. Surfactant production levels can affect bacterial adhesion to oil droplets because of lowered oil-water interfacial tension. These contending effects, (i.e., increasing bioavailability/interfacial area and decreasing surface adhesion of bacteria) mean optimum surfactant application for the cleanup process must be carefully considered [41] mean optimum surfactant application for cleanup process must be carefully considered and controlled if the need be [41]. Bioavailability is of great importance in initiating a bio-remediation process and could be the rate-limiting factor when bioavailability is poor. The action of biosurfactants increases bioavailability and, in turn, the potential for biosorption (a process where microorganisms use their cellular structure to capture nutrients, which they can sorb onto the binding sites of the cell wall). Because petroleum components have high hydrophobicity and low polarity, they remain tightly associated with soil particles and are therefore not readily bioavailable for degradation by the hydrocarbonoclastic microbial community [42]. Currently, different methods have been developed over the years and have evolved into strategies applicable to the bioremediation process. In the case of the unassisted remediation process, the remediation solely relies on the microbial community's strength and the contaminants' bioavailability. The advantage of bio-stimulation over natural soil attenuation is that there are basic required nutrients (macro and micro nutrients) aside from carbon that are necessary for microbial survival. The stimulants not only arouse the degraders but also make some of the deficient nutrients available to the microbial community. Previous researchers have pointed out carbon dioxide (CO_2), methane (CH_4), and water vapor ($H_2O_{(g)}$) as by-products of the biological degradation of petroleum. However, these greenhouse gases (GHGs) are produced in insignificant quantities to contribute to the greenhouse effect and hence cannot be accounted for as a disadvantage in applying this method.

Ecotoxicology and Empirical Studies on PHC Bioremediation

Adeleye [43] studied the effect of microorganisms in the bioremediation of spent engine oil and petroleum-related environmental pollution and investigated the effectiveness of using hydrocarbonoclastic microorganisms (bacteria and fungi) in biologically remediating soil polluted by the pollutants showed that: Klebsiella aerogenes, Klebsiella pneumonia, Pseudomonas alcaligenes, Bacillus coagulans, and Pseudomonas putrefaciens exhibited a soil degradation rate of spent engine oil, whereas fungi, such as Aspergillus, Cephalosporium, and Pencillium species, were found to be the potential microorganisms responsible for hydrocarbon bioremediation. The study focused on the effect of the natural strength of microorganisms in soil attenuation without bio-stimulation or bio-augmentation. Hydrocarbon oxidizers must meet certain requirements to accomplish the task of bioremediation. Therefore, the degrading microbes provided with the optimum environmental conditions and basic and adequate nutritional requirements will bring about the desired results in the remediation process.

Regarding the bioremediation of crude oil-contaminated soil, Nnadi and Osakwe [44] used livestock droppings of poultry and cows to investigate their effect on the rate of remediation compared with that of naturally attenuated soil. The total heterotrophic count was taken for 150 days, and the results showed that contaminated loamy sand had $3.0x10^7$ cfu/g and contaminated clay soil had $2.2x10^7$ cfu/g. Contaminated loamy soil, cow dung, and poultry dropping had $3.0x10^7$ cfu/g, contaminated clay soil, cow dung, and poultry dropping had $2.0x10^7$ cfu/g, contaminated clay and cow dung had $2.0x10^7$ cfu/g, and contaminated loamy and poultry dropping had $3.2x10^7$ cfu/g, while contaminated clay and poultry dropping had $2.1x10^7$ cfu/g. The research showed that the bioremediation of crude oil is enhanced more in loamy sandy soil than in clay soil when using organic fertilizers such as cow dung and poultry droppings, although information about the type of degrader was not provided. When comparing sites contaminated with weathered hydrocarbons, the toxicity of a freshly contaminated site is high due to the presence of toxic lower boiling-point fractions, such as the C_{10}-C_{19} range [45, 46]. At a certain period during remediation, the hydrocarbon contaminant concentration stabilizes, and this value is termed as the residual or irreducible concentration.

Chorom et al. (2010) [47] investigated the bioremediation of a crude oil-polluted soil by applying fertilizers by bio-stimulation. They applied the strategy of actively aerating the soils and adding fertilizer (N, P, K) to promote oil biodegradation by indigenous microorganisms. Artificially polluted soil and then fertilizers were applied in 3 levels of 0, 1, and 2 ton/ha in 3 replicates. The soils were kept in 30 °C and 60% of the field capacity for 5–10 weeks. The results indicated that the applied fertilizer increased the degradation of the hydrocarbons compared with the control. In addition, the application of fertilizers at a rate of 2 ton/ha in oil-contaminated soil led to greater rates of biodegradation after 5 weeks, indicating the feasibility of bioremediation. However, the lack of organic feeding matter limited the oil degradation and led to an increase in the C/N ratio.

The efficiency of cassava steep liquor (CSL) in bioremediation of diesel oil-contaminated tropical agricultural soil was investigated. The soil was contaminated artificially with diesel oil and treated with CSL, which was designated EXPS by Adebusoye [48]. Similar polluted soil without CSL amendment (CSS1) and uncontaminated soil (CSS2) served as controls. The investigation showed dramatic changes in the physicochemical properties of the EXPS and CSS1 when inorganic nutrients were utilized to the tone of near-depletion in the former than the latter. Physicochemical properties changed drastically in stimulated soil than in naturally attenuating soil, but only the physicochemical properties (pH, TPH) of the soil were considered without the biological properties. The use of field cells in bioremediation of petroleum-hydrocarbon-polluted agricultural soil at various levels of soil tillage has indicated that soil petroleum contamination has depleted oxygen reserves and slowed down the diffusion rate to layers of greater depth [49]. The application of mineral fertilizer and at different rates of aeration through different soil tillage levels with conditions of a major spill simulated by sprinkling petroleum on the cells and soil physicochemical characteristics monitored at intervals of time showed that the TPH content reduction (88%–99%) in the treated cells was significantly different from the control. These results support the view that the accelerated biodegradation rate of PHCs in polluted agricultural soils is induced by the availability of large amounts of oxygen in the soil profile. However, regular contaminated soil tillage with sufficient nutrient content could achieve rapid soil decontamination.

Bioremediation is a technique that exploits the inability of petroleum to resist microbe attacks. The ability of certain microorganisms to utilize PHCs as a carbon source in their metabolism has been proven for about 80 years. Bioremediation is a chemical oxidation/reduction technology that is achieved by modifying the chemical composition of the pollutant from reagent addition, thereby increasing the degradation and extraction of contaminants and converting them into less toxic, less mobile, or inert compounds. Surface tension can be lowered by the secretion of biosurfactants (such as rhamnolipids) by biosurfactant-producing degraders isolated from the native desert soils used in bioremediation of petroleum-contaminated soils [50]. Bioremediation has proven to be effective not only in the remediation of terrestrial habitats but also in cases of marine spills. On account of the 1989 Exxon Valdez Oil Spill, a tragic event that specifically pointed out to the United States and the world at large the lack of preparedness the petroleum industry had downplayed in cases such as petroleum pollution and environmental contamination. However, this tragic event instigated and triggered further research that explored the use of living organisms for remediating marine oil spills—an effective method promoted by its eco-friendliness and effectiveness [51]. There are many downsides involved in the use of bioremediation in marine spills due to the difficulty in concentrating spills in water.

Ofoegbu et al. [2] investigated the bioremediation of crude oil-contaminated soil using organic fertilizers (cow dung, palm kernel ash) and inorganic fertilizers (NPK) as bio-stimulants for a 40-day period under laboratory conditions described from the application of the first-order kinetic model. The results showed that soils amended with organic fertilizers had a higher degradation rate constant (k) and lower half-life than other bio-stimulants in the experiment. Judging from the estimated bio-stimulation efficiency (B.E) and biodegradation rate constant (k), the remediation order from the best treatment regime adapted was as follows: (inorganic fertilizer + cow dung) > (inorganic fertilizer) > (cow dung) > (cow dung + palm kernel husk ash) > (palm kernel husk ash) at 2.4% crude oil contamination, and inorganic fertilizer preceding a combination (inorganic fertilizer + cow dung) at 6% crude oil contamination.

Relatively, in terms of total microbial count, the works of [2] showed higher growth in contaminated loamy soil amended with cow dung than that of poultry. The results showed that bioremediation is enhanced more in loamy sandy soil than clay soil using organic fertilizers of cow dung and poultry droppings. However, the quantitative contribution of the addition of the stimulants on the microbial community population was not provided.

Petroleum oil contaminants have become severe ecological problems and negatively impact human health. Therefore, identifying environmentally friendly approaches to remediate oil-polluted environments is imperative. El-Lithy et al. [53] assessed bacterial oil degradation stimulated with a nitrogen source under optimum conditions. Strain ODB H32 was acquired from oil-based mud of selected oil-drilling sites in the western deserts of Egypt and was identified using 16S rRNA analysis. Accordingly, using BIOLOG GEN III, the metabolic fingerprints of E. hormaechei revealed that the bacterial strain is capable of using diverse carbon and chemical components; nonetheless, it was able to degrade 0.6% of oil contamination under optimum conditions of 7.0 pH and 30^{o}C temperature. In addition, the analysis revealed that nitrogen stimulants were good growth enhancers in the process, with peptone being a better enhancer than yeast, yeast being a better enhancer than ammonium nitrate, and ammonium nitrate being a better growth enhancer than urea in the growth of E. hormaechei on MSM. Capillary gas chromatography revealed a maximum degradation of peptone (70.7%) by E. hormaechei, indicating that peptone is a good biostimulant for petroleum degradation. These findings imply that the use of biostimulated E. hormaechei as an eco-friendly approach for remediating oil-polluted environments under optimized conditions, especially in arid regions like the western desert of Egypt. Nonetheless, nutritional requirements may be diverse for different microbial organisms.

Felix et al. [54] investigated the bioremediation of diesel-polluted soils with water hyacinth and assessed the efficacy of both fresh and powdered water hyacinth as potential bio-stimulants in the remediation of diesel-polluted soil. Three test concentrations (50g, 100g, and 150g) and a control (0g) were prepared and monitored by assaying the TOC, TPH, and soil pH for 90 days before and after the introduction of fresh and powdered water hyacinth. The results before the introduction of water hyacinth showed an increase in soil pH, total organic carbon (TOC), total dissolved solids (TPH), and volatile matter when compared with the control due to soil contamination by diesel oil. However, at the end of 90 days, experiment fresh water hyacinth produced better results in remediating polluted soil than powdered water hyacinth.

Even though the soil acts as a natural reservoir for contaminants, the need for a special intervention process to reduce these contaminants to a safe level or completely exterminate them has become inevitable [55]. One promising approach to enhancing the bioavailability of petroleum hydrocarbons is the application of surfactants [56, 57], which seeks to increase the solubilization/emulsification of petroleum hydrocarbons by enhancing their dissolution or desorption rates. Bacillus sp. DQ02 adherence to hydrocarbon increased by 44% in the presence of Rhamnolipids [58], with increased removal of n-hexadecane by 11.6% more than when Rhamnolipids were deficient. However, reports have noted that some surfactants (e.g., Corexit 9500), on the contrary, negatively impacted the hydrocarbonoclastic bacteria community [56] because of the toxic nature of the surfactant toward bacteria and due to contention over HC substrates. On this account, the selection of a suitable and appropriate surfactant is of great necessity to impede further environmental pollution [59]. Generally, bio-emulsifier-producing bacteria that have drawn much attention have been found to collectively exhibit the following two physiological attributes:

1) The ability to enhance the complexation and solubilization of non-polar substrates and consequently promote the bioavailability of substrates;

2) Ability to improve the affinity between cell surfaces and oil-water interfaces through biological processes, promoting the deformation of the oil-water interface film.

Bacillus amyloliquefaciens produces a bio-surfactant that is a better substitute for chemically produced surfactants. It exhibits a high solubilization efficiency toward diesel oil (71.54% at 1 g/L), which is better than sodium dodecyl sulfate (also known as sodium dodecyl sulfate or lauryl sulfate) and polyoxyethylene sorbitan monooleate (also known as polysorbate 80 or Tween 80), and is capable of enhancing the efficiency of diesel-oil degradation of the An6 strain [60]. Nonetheless, this is not the case for all bio-surfactants produced by bio-emulsifier-producing bacteria because not all can effectively enhance the rate at which the degradation of pollutants proceeds [61]. Whether various bio-surfactants stimulate or inhibit the bioremediation of pollutants depends on the physicochemical properties of the surfactants, types and characteristics of pollutants, and physiological characteristics of the functional microorganisms [62]. Thus, establishing a database of petroleum hydrocarbon contaminants and bio-emulsifier-producing bacteria that is conducive to the targeted selection of bacteria suitable for the treatment of petroleum hydrocarbons is necessary.

Trejos-Delgado et al. [63] studied the effect of Tween 80 surfactant in the biodegradation of PH-polluted soil (obtained from the Colombian Amazon forest region) in the laboratory and pilot-scale under aerobic conditions. Tween 80 was used in different doses with LE distributed in four biostimulate treatments, a control treatment, and natural attenuation treatment. The effect of organic stimulators and nonionic surfactants was assessed at the laboratory and pilot-scale levels. The pilot-scale study was conducted in a passive aeration reactor, and the better performance treatment test was conducted at the pilot-scale in a convective flow reactor (CFR). Treatment that included LE and Tween 80 (1.5 g/L) gave the best result with 52% TPH removal in 80 days, an indication of the improvement the bioremediation treatment regime had on natural process of degradation. Tween 80 promoted TPH solubility, as seen in the increased rate of CO_2 by-production from the process in distinctive bioremediation treatments in both periods. The kinetics of CO_2 production showed that the system required a periodic addition of a co-substrate and an increase in soil microbiota through the addition of compost (pilot scale). In this stage, more than 76% of the contaminant was degraded in 60 days.

Y. Waychal et al. [23] pointed out a research gap on microbial matter; remediation techniques focused on petroleum degradation methods and parameter optimization, with parameters such as soil nutrient (e.g., nitrogen, phosphorus, and dissolved oxygen concentrations), augmented petroleum-degrading microbial community, and environmental factor impact, being studied for biological remediation efficacy [64]. A study by Martínez Álvarez et al. [65] showed that adjusting the level of phosphorus, carbon, and nitrogen with the development of a pilot scale for diesel-contaminated soil. The resultant diesel degradation efficiency was 54.9% when the carbon (C), nitrogen (N), and phosphorus (P) ratio in the soil was adjusted to 100:17.6:1.73, whereas the unadjusted ratio of 100:10:1 gave a diesel degradation efficiency of 27.8%, confirming the point of parameter adjustment.

Furthermore, the technique of anaerobic bioremediation is a cost-effective method that permits very little to zero oxygen concentration at remediation sites; a technique that has recently gained the attention of various researchers [64]. In the application of the anaerobic bioremediation technique, oxygen is substituted for chemical components such as nitrates, sulfates, iron, and carbon dioxide by microorganisms.

Alvarez et al. [66] investigated oil pollution in the arid climate of the Republic of Kazakhstan and developed a new biopreparation (consisting of bacterial strains R. qingshengii F2-1, R. qingshengii F2-2, and P. alloputida BS3701) for soil cleanup. In a period of 50 days, laboratory studies showed 39% PAH and 48% n-alkane degradation using biopreparation in a liquid mineral medium of 15% petroleum. The effectiveness of biopreparations can be seen in the field experiments conducted at the K-Kurylys landfill of the Republic of Kazakhstan on soil contaminated with 10% petroleum over a period of six months. The experimental results revealed that the concentration of oil degraders reached 107 cfu/g of soil with a TPH removal efficiency of 70% at the end of the experiment.

Zhang et al. [67] investigated agricultural wastes, such as wheat bran and swine wastewater, for the bioremediation of oil-contaminated soil using two selected optimized strains (A and B) that could efficiently degrade oil. They showed that the best ratio of strain A (Bacillus subtilis CICC 21312) to strain B (Candida bombicola ATCC 22214) was 7:3, indicating that swine wastewater could be a replacement for nitrogen source and process water for bioremediation.

After 40 days, the rate of oil degradation increased to 68.27± 0.71% under the optimal medium: microbial dosage (97 mL/kg), swine wastewater (232 mL/kg), and wheat bran (158 g/kg) implementing the Box-Behnken design for culture medium optimization. However, comparing the degradation rates of different methods, Zhang et al. [67] outperformed Besalatpour et al. [68], who employed a land tillage technique to remediate 500kg of soil contaminated with petroleum oil, resulting in a TPH decrease of 50% after a period of four months. They also outperformed Wu et al. [69], who adapted the technique of mixed culture degradation (i.e., the use of mixed degrading bacteria) to restore soil initially contaminated with 82,533 mg/kg of TPH content, decreasing the TPH content to 47,600 mg/kg (with a TPH removal efficiency of 42.3%) in a period of 13 weeks. The approach of Zhang et al. [67], compared to Besalatpour et al. [68] and Wu et al. [69], performed better in terms of remediation time shortage and further solved the problem of piggery wastewater pollution in the process. These investigations might lay a foundation for reducing the pollution of agricultural wastes, providing a promising means for W2W conversion.

Traditionally, the toxicity of petroleum hydrocarbon contaminants in the soil can be estimated based on chemical fractions and a range of bioassays including invertebrates, plants, and microorganisms [70]. In the works of Tang et al. [71], soil samples contaminated with petroleum hydrocarbons collected from Shengli Oilfield of China were analyzed for toxicity based on earthworm acute toxicity, plant experiment, and luminescent bacteria test. The TPH concentration of soil contaminated at 10.57% with lethal and sub-lethal rate endpoints gave results inferring that the TPH concentration at 1.5% is critical for plant growth and earthworm living, and the activities of luminescent bacteria will be affected at 0.5% concentration. Controlling petroleum hydrocarbon contamination in aquatic habitat is more difficult than in terrestrial habitat. Crude oil in water results in the formation of a hydropholic layer, a crude oil barrier between water and air, thereby causing a decline in aeration and a decrease in life span in both terrestrial and aquatic habitats. Oil pollution has caused great harm and destabilized the natural ecological system [71]. For example, the Gulf of Mexico deep water horizon oil spill accident, which produced a profound, long-term socio-economic impact on the environment, is still a global focus. Although there is a growing concern about the toxic effects of petroleum pollution on living organisms in affected areas [72, 73], the severity of its toxicity on affected microbial communities is often unmarked. The greatest negative effects of petroleum hydrocarbons inhibited microbial biomass in gasoline-polluted sand soil [74]. Research has found that the effects of diesel fuel toxicity resulted in decreased species richness, evenness, and phylogenetic diversity, with the resulting community being dominated by a few species (principally Pseudomonas) to a great extent. Furthermore, the decline in phylogenetic richness and diversity was linked to nitrogen cycle disruption, with a significant reduction in species and functional genes that play a role in nitrification [75, 76]. The toxicity of 1-methylnaphthalene, 2-methylnaphthalene, and naphthalene, as well as their oxygenated derivatives, to Agmenellum quadruplicatum bacterial cells has been found to produce no significant inhibitory effects on bacterial cell growth. However, quinonic and phenolic naphthalene derivatives inhibit bacterial cell growth. This could be explained by the higher solubility of phenols and quinones, which enhances the mass transfer of molecules to bacterial cells, resulting in higher toxic effects than the former compounds. Reported studies have shown that some metabolic intermediates produced from the degradation of petroleum by microbes (bacteria), having relatively high solubility, may have greater cytotoxicity than their parent molecules, resulting in cell damage [77]. However, indigenous bacteria form extensive aggregates with each species performing specific functions. While some bacteria sensitive to petroleum hydrocarbons are inhibited upon exposure to petroleum and its contaminated environs, those efficient degraders of petroleum hydrocarbons, as well as those resistant to cytotoxic metabolites, will flourish in such polluted environs. It is understandable that the presence of toxic matter in an ecosystem damages the ecosystem and denaturing microorganisms in the environment. However, the degree of ecotoxicity (coupled with other factors) determines the level to which the bioremediation capacity and capability of the native/indigenous microbes are weakened and the degree to which stimulation will be quintessential.

Ecotoxicity and its Effect on the Phase Change of Microbial Growth

There are four progressive phases in microbe growth:

- **Lag Phase**: This stage is at the beginning of the process where there is no observable increase in the microbial community population, i.e., latent growth.
- **Log/Logarithmic/Exponential Phase**: There is a noticeable active growth and an increase in the microbial community size.
- **Stationary Phase**: At this stage, the rates of mortality and natality are slightly balanced, and the size of the microbial community appears to be unchanging.
- **Death phase:** In this stage, there is a gradual decrease in the growth rate of the microbial community. In other words, the mortality rate increased over the natality rate.

Bio-augmentation may speed remediation efforts in areas where the current size of indigenous microbial communities is not capable of degrading all hydrocarbons present, or where native species are in extended lag phases due to oil exposure [28, 78, 79]. The longevity of the log phase of microbial growth is limited by the degree of toxicity of the polluted environment because the necessary conditions for growth are made latent or dysfunctional by their presence. Hence, the log phase may be skipped in a bioremediation process if environmental conditions are not favorable to support microbial growth. The assimilation of petroleum contaminants by microorganisms is a quality mapped out by various taxonomic groups [80]. Research has shown that in habitats plagued by oil contaminants, the most common species of microorganisms are bacteria of the genera Rhodococcus, Arthrobacter, Pseudomonas, and Acinetobacter [81]. Many scientists have actively studied Pseudomonas and Acinetobacter for environmental remediation because of their ability to degrade a diverse number of intoxicants in the environment [82, 83, 84, 85, 86]. The potential toxicity effects of pollution on soil microorganisms usually result from the vulnerability of soil microbiota to various levels of pollution that they cannot effectively dispel. This results in a substantial change in the different microbial processes associated with bionomic services. Although the toxic effect of pollutants in the soil is well known, very little is known about the effects of different organism groups present in the soil. Continuous deposition and accumulation, slowed degradability, and longer persistence of pollutants in the soil adversely affect the community size of beneficial soil organisms. Toxicity assessment has been based on maximum tolerable concentrations of pollutants in an ecosystem. The strength of bioremediation is to a large extent dependent on the size of the remediating community. Therefore, the sole reliance on the strength of indigenous microbial communities for the clean-up of affected areas will span longer periods. Hence, interventionary measures must be developed to accelerate degradation processes. Puntus et al. [87] investigated the physiological, biochemical, and metabolic properties of petroleum-degrading microorganisms. PAHs are some of the most toxic pollutants of petroleum origin. According to Nuerla et al. [6], the WHO standard PAH concentration is extremely high at concentrations (≥ 1ppm). PAHs (and other by-products from degradation) are highly cytotoxic compared to alkanes [87]. Aliphatic compounds (e.g., diesel and grease) are more amenable to biodegradation than aromatic compounds, and the higher the number of rings, the more difficult the biodegradation [16].

Recently, the biological degradation of aromatic hydrocarbons has been achieved by biological stimulation of the involved bacterial strains [89]. Bayha et al. [90] reported that the decreased invulnerability of fish (southern flounder) to bacterial (Vibrio anguillarum) infestation was counterchallenged on exposure to petroleum. The fatality rate of the fish exposed to petroleum was heightened (94.4%) even before their encounter with bacteria, and mortality (<10%) was observed for fish unexposed to petroleum before bacteria infestation. Owing to the nature of the oil industry, exposure to hydrocarbons (mainly PAHs) is accompanied by various symptoms, such as confusion, vomiting, nausea, skin irritation, and mental disbalance. Nevertheless, information on the precise biological mechanism by which such health-related challenges are triggered on exposure to PAHs is inadequately available [91, 92]. Many carcinogens that affect humans have been found to be of petroleum origin, considering reports that have traced the cause of respiratory cancer to inhalation of PAHs. In fact, in 2018, the International Agency for Research on Cancer (IARC), France, in 2018 declared that emissions from various petrochemical industries have carcinogenic characteristics. Cognitive research studies have discovered that the morbidity of cancer diagnosis was predominant in areas not far from petroleum exploration and refineries [93]. Petroleum contaminants in soil can alter its physicochemical properties, such as mineral nutrients (such as sulfates, sodium, phosphate, and nitrate), pH, permeability, and organic carbon, which directly affect the ontogeny of soil micro- and macrobiota [94, 95, 96, 97, 98].

Synthetic Biology: Bioremediation Genetic Engineering

Some of the basic established methods (thermal, chemical, physical) employed in restoring petroleum-polluted sites are usually costly, time-inefficient, and require human effort to operate sophisticated machineries, which could also lead to extensive environmental pollution. This is why researchers have encouraged the method of bioremediation, an ecofriendly means with the lowest possible risk of provoking secondary environmental pollutions. Conventional bioremediation uses native/indigenous bacteria in the soil to achieve remediation. However, bioremediation is an ecologically friendly means that also has its downsides. Recent research has shown that many microbes are incapable of breaking down a few of the most stubborn and dangerous xenobiotic chemicals, i.e., chemicals (synthetic or unnatural) that may persist in the environment over a long period of time due to their intricate organic structure.

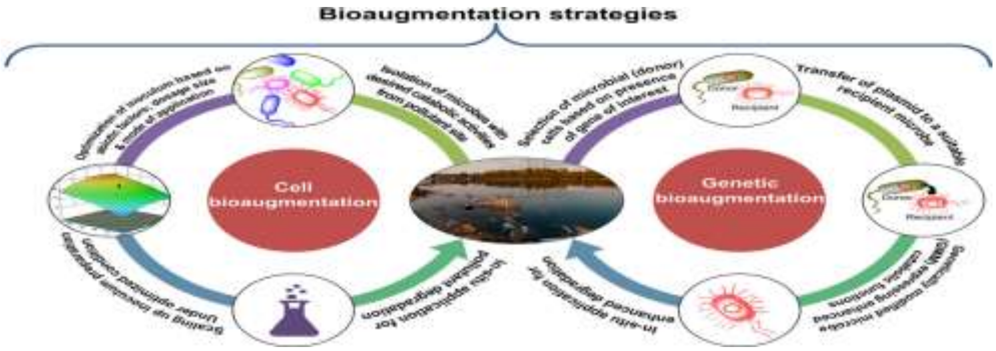

Figure 8: Bioaugmentation strategies

Xenobiotic chemicals, such as insecticides, explosives, and strongly nitrated or halogenated arenes, are chemically inert under natural circumstances and are therefore not properly decomposed [99]. These complexities arising from pollutant combinations, coupled with the toxicity effect to indigenous microbial communities, have reduced the possibility of a successful remediation process even with the practice of usual cell bioaugmentation [100] (Figure 8). However, research has shown that inculcating scientific, biological, and engineering principles with the aim of redesigning and restructuring the genetic makeup of living organisms to optimize or create new biosystem with enhanced characteristics [101, 102, 103], is achievable in the field of synthetic biology. Synthetic biology involves the use of molecular tools and biological systems in reprogramming genetic frameworks and building and rebuilding synthetic pathways with the goal of producing living organisms (microorganisms) with advanced and unconventional characteristics [102, 103]. It is also known as genetic engineering and sometimes as bioengineering. Improved species have been created by manipulating the DNA of oil-degraders, as various genetic methods have been developed and employed in optimizing enzymatic and metabolic pathways of organisms with regard to biological degradation, which has been widely applied in genetic bioaugmentation for environmental resuscitation [104, 105]. Although the use of indigenous microbial communities in remediation is effective, nonetheless, in other to fix the problems encountered by rate limiting factors, indigenous isolates are modified into recombinant microbial strains with possibly greater potential for oil degradation than their ancestors. The ability to readily alter sequences and genetic activities in organisms can help tackle these challenges. Therefore, bioremediation using modified bacterial strains is considered an option in oil contaminant elimination [106, 107] considering the role of microbial community-soil interplay on substantial hydrocarbon decomposition [108, 109]; a basis for extensive research. The essence is aimed at strategically designing novel strains capable of degrading high molecular weight polyaromatic compounds. The first genetically engineered microorganism (GEM) that was approved for field testing in the US for biological remediation process (i.e., Pseudomonas fluorescens HK44), also known as the HK44 strain, has an introduced lux gene that serves as a reporter gene (i.e., a protein-coding gene that is frequently tagged to a gene of interest) coalesced within a naphthalene degradative pathway, which hereby permits recombinant microbe to exhibit bioluminescence as specific PAHs (e.g., naphthalene) are being degraded. This is a fascinating phenomenon as cell density and the bioremediation process are made observable by light detection. However, studies have ascertained that strains of bacteria capable of digesting various pollutants, such as nitro-aromatics, chloro-aromatics, and polycyclic aromatic compounds, have been distinguished for their metabolic capability to biologically remediate polluted/contaminated areas [110].

Anand Mohan Chakrabarty, an Indian-born American microbiologist, pioneered the development of the oil-eating ‘superbug’. Using four Pseudomonas strains containing plasmids (a minute circular DNA molecule found in bacteria, physically separated from chromosomal DNA and capable of independently replicating itself), isolated from each strain capable of degrading octane, camphor, naphthalene, and xylene, he subsequently introduced all four plasmids into a single strain of Pseudomonas putida by modifying the superbug strain into one capable of degrading octane, camphor, naphthalene, and xylene. Considering the complexity of hydrocarbons, the use of plasmids in degrading diverse crude oil constituents in a single cell is a welcomed technology in the creation of superbugs given that the extra-chromosomal plasmid has control over degradation of particular crude oil components by microorganisms.

Zakaria et al. [111] modified the chromosome of A. baumannii S30 by introducing its lux gene (a gene that encodes bacteria luciferases: a bioluminescent oxidative enzyme) into an Acinetobacter baumannii S30 pJES strain whose presence is tractable as a result of its persistence in bioremediation (Figure 9).

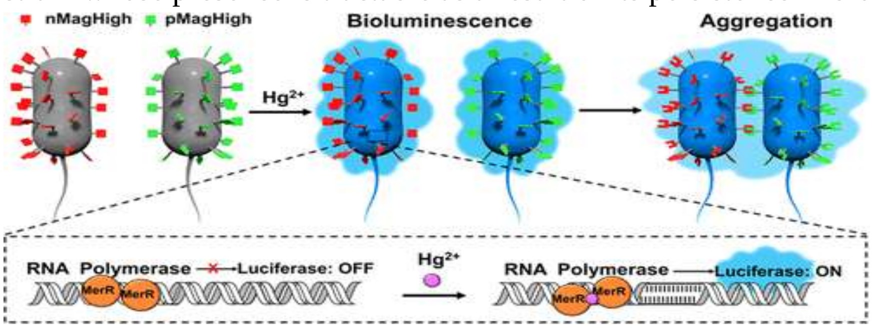

Figure 9: Bioluminescence of luciferase

Ajona et al. [107], investigating on ‘bioremediation of crude oil-contaminated soil using recombinant native microbial strain” and focused on the biodegradation of n-alkanes in artificially oil-polluted soil by micro-organic strains. In his approach, two selected indigenous isolates from five isolates were used in an explorative study of their potential biodegrading ability. The strains isolated from petroleum contaminated soil gathered from the locations of close proximity to petroleum wells in Thiruvarur district, Tamil Nadu, India. Of the two selected isolates for recombinant progression, one promising isolate was inoculated with the wild microbial strain at the end of the recombinant procedure at ambient conditions at various petroleum concentrations (0.5%, 1%, 3%, and 5%) for a period of 42 days. TPH analysis was carried out and tracked by GC-MS analysis, and TPH removal was more enhanced in contaminated soil inoculated with the modified strain than in the wild strain at the lapse of 42 days. However, the study pointed out that out of over 50% of the native oil-degrading bacteria colonies isolated one step ahead, one of the oil-eating bacteria with a high potential for oil degradation was isolated for study.

Genetic engineering has been applied in the modification of metabolic pathways by which microbes transform oil contaminants into useful products, such as polyhydroxy alkanoate (PHAs) [112]. Experiments have shown that metagenomic clones and host cells are made to carry the genetic material of microorganisms from oil contamination sites through genetic modification. Dellagnezze et al. [113] employed a bioaugmentation strategy in a microbial consortium immobilized in chitosan beads for oil degradation in mesocosm to study alkane degradation inoculated with clones of oil-degrading Bacillus subtilis in a laboratory culture. At the end of 30 days, the removal efficiency was 85% with inoculated clones, whereas the wild-type control had a PAH removal efficiency of 68% [113]. In another case, Vasconcellos et al. [114] experimentally screened for hydrocarbon biodegraders in a metagenomic clone library sourced from petroleum reservoirs in Brazil and used metagenomic clones of Escherichia coli (E. coli) host cells in a DNA fosmid vector consisting of approximately 40kb of unspecified environmental DNA. E. coli do not favor adherence to HCs, which consequently discourages bioremediation because they are hydrophilic [115]. Studies have also shown that fumigants have a xenobiotic component called 1, 2, 3-trichloropropane [116], which in E. coli is dissipated into the environment by a process known as heterologous catabolism, a combination of enzymes from two or more distinct microbes. On the other hand, Psylvania Sp. has a broad empathy for HCs and can degrade selected alkanes, thiophenes, and alicyclic and aromatic HCs [107]. To encourage oil droplet-cell attachment, the hydrophilicity of the cells must be overcome. However, Wang et al. [117] integrated synthetic modules to model an E. coli strain with phenol-to-carbon conversion capability. Selected phenol-degrading genes were isolated and modified, and two metabolic modules were constructed using two phenol hydroxylase genes and seven catechol-degrading genes. They were both incorporated into a vector for E. coli cell transformation. Engineered strains degraded phenol rapidly in wastewater contaminated with 5 mM petroleum in 7 h, demonstrating a novel metabolic pathway for the successful phenol eradication of E. coli. Bacteria are naturally incapable of phenol degradation. However, Wang et al. [117] synthesized a modified strain capable of not only degrading phenol but also converting it into a usable carbon source. Catechol 2,3-dioxygenase encoded by xylE is an important enzyme that facilitates the biodegradation and biotransformation of arene molecules [118]. Acinetobacter sp. BS_3 (a strain cloned originally from Acinetobacter sp. $BS_3C_{23}O$) had a broader scope of substratum particularity in n-alkanes and arenes degradation than its ancestor species, when xylE cloned from plasmid DNA was expressed in its chromosome. This development demonstrates the important role of plasmid selection in the genetic engineering of microbes for the creation of new metabolic pathways that promote oil-contaminant sequestration and elimination. Plasmids can carry degradative genes that encode enzymes necessary for the degradation of aliphatic, aromatic, and PAHs [118, 119]. Horizontal gene transfer (HGT) of bacterial plasmids may be facilitated by three mechanisms: transformation, transduction, and bacterial conjugation. The mechanism of transformation is widely preferred over others in introducing choice genes into another microbe using different vectors [119, 120, 121]. The PHE plasmid contains genetic information necessary for phenol metabolism, and the TOL plasmid encodes xyz genes that are necessary for the degradation of toluene and xylene. Naphthalene catabolic genes are part of the NAH plasmid, and the OCT plasmid is responsible for octane degradation by alk genes [118, 119, 122]. These four are the most commonly used plasmids in microbial engineering for recovering hydrocarbon-contaminated sites. Genetic optimization can be performed to obtain the best genetic platform to maximize metabolic efficiency, on the other hand some of these methods involve the search for homologous genes or the optimization of codons (a DNA or RNA sequence of trinucleotide in a unit form) to match the host or to find the most befitting degradation genes for the chosen microorganisms. It is also possible to regulate the number of plasmid copies and mRNA expression and to modify the translation rate by modifying the RBS [123]. All of these modifications could have different effects, such as minimizing chokepoints, avoiding hyper-expression of an enzyme, or increasing the affinity or binding of the ribosomes to the transcripts [124, 125]. Gene expression can be regulated by many factors, thus fine-tuning enables the achievement of a proper balance in pathways to maximize metabolic efficiency. Jain et al. [126] studied protein (enzyme) involvement in HC degradation by in silico analysis. In this analysis, an orthologous gene for the enzyme monooxygenase (also known as ladA) was discovered in the Burkholderia thailandensis MSMB121 strain, and different ladA amino acids were replaced by employing homology modeling in its structural characterization. However, an observable advancement in the binding energy to different alkane chains was achieved, with a resultant substrate bioavailability and creating a foundation for further substantiation and proof of HC degradation pathways in B. thailandensis [126]. This study has shed more light on the claim that genetic optimization as a tool to attain metabolic pathway alteration and improvement is achievable for complex HC mixture degradation.

Safety and cost-effectiveness of employing engineered microbes rather than alternative methods [127]. Genetically engineered microbes (GEMs) have proven to be better degraders of petroleum contaminants and diverse pollutants such as xylenes, toluenes, naphthalenes, halobenzoate, tricholoethylene, and octanes because of their ability to quickly detect pollutants and adjust and adapt to changes in toxicity, which may be due to the introduction of new pollutants encountered or co-metabolized by them. However, it is uncommon knowledge that bioaugmentation has arisen as a resolution to the challenges of sulky and inert decomposition of pollutants when GEM strains are employed in pollutant degradation [128, 129, 130, 131]. The use of genetic engineering to improve the inherent remediation capabilities of microorganisms is becoming popular [132, 133] and gaining wide acceptance in the science and engineering space. Atomistically and wholistically considering the aspects that affect degradation rates is necessary to gauge the rate of contaminant degradation. Considering that oil-degrading microbes exist in the environment alone is not enough to judge the flow of a remediation process. However, contaminant degradation is abated/disturbed in a competitive circumstance. Under favorable conditions, non-hydrocarbonoclastic bacteria are likely to gain favorable circumstances that will encourage an exponential increase in their size, suppressing the growth of oil-degrading bacteria as a result of competition for nutrients, eventually becoming the leading species in the surroundings [129,130]. Understanding metabolic pathways can be improvised in solving issues such as nutrient competition, which may arise from the use of GEMs. Understanding metabolic pathways is important in the study of microbial bioremediation, such as bioremediation toxic contaminants by the production of haloalkane dehalogenases and the decontamination of pyrethroid from soil via the fenpropathrin biodegradation mechanism deliberated in Bacillus sp. DG-02 [132]. Conventional bioremediation has been greatly improved through the deliberate alteration of metabolic routes, laying a foundation for the extensive study of enzymes (such as oxidases, esterases, monooxygenases, oxidoreductases, and phenoloxidases) and enzymatic pathways [133]. Enzyme-based bioremediation offers numerous benefits. For example, the enzymatic action of esterase D on endosulfan (an organochorine pesticide) yields simpler molecules, thereby increasing substrate bioavailability. Recombinant enzymes are more likely to be synthesized via genetic techniques. White rot fungus secretes enzymes capable of breaking down polycyclic aromatic hydrocarbons (PAHs), 2,4,6-trinitrotoluene (TNT), and polychlorinated biphenyls (PCBs). In addition, a LiP-encoded enzyme (Phanerochaete chrysosporium hemoproteins) has been found to be a good PAH decomposer [134].

Enzyme immobilization significantly improves half-life, stability, and enzyme activity. Efficient and environmentally acceptable enzymatic bioremediation of persistent xenobiotic substances may be achieved using this approach [135]. However, the presence and concentration of various pollutants other than PHs can interfere with soil biological and co-biological processes [136], such as the concentration of heavy metals and other chemo-toxins in the area. The use of GEMs could solve the challenges encountered in the degradation of uncompromising and xenobiotic toxins. Further research has shown that despite being flora, yeast and fungi are also efficient petroleum eaters [137, 138]. Das and Chandran [139] reported that the biodegradation efficacy ranged from 6% to 82% for soil fungi, 0.13% to 50% for soil bacteria, and 0.003% to 100% for marine bacteria. The major hydrocarbon-degrading microbes were from the genera: Alanivorax, Bacillus, Pseudomonas, Bravibacillus, Acinetobacter, Dietzia, Methylobacterium, Caulobacter, Halomonas, Flavobacteriales, Candidauts, and Vibrio [140, 141, 142].

Genetic engineering of microbes has previously been considered as a remediation technique, but the long-term implications on indigenous species and the larger effects on the marine ecosystem due to the supplementation of genetically modified organisms have not been conclusively determined because less consideration has been given to field needs and other difficult scenarios that may arise later in the process of designing bacteria. To overcome this challenge may be the use of “suicidal” genetically engineered microorganisms (S-GEMS) programed to die after the contaminant is eradicated [27]. The strategy of designing S-GEMS is based on the knowledge of killer-anti-killer gene(s)–gene(s) that will be susceptible to programed cell death after the detoxification of any given contaminated site [143]. Self-destruction mechanisms (vectors or suicide genes) may be included in these genetically modified organisms (GMOs), or they may only be viable under specified environmental circumstances for which they were deliberately engineered [144]. To control and observe the spread of GEMs in the environment and possibly mitigate their activities at some point, tracking them is essential. This has made it possible to create a linkage between target and reporter genes under a promoter control. Remediation process is monitored by reporter gene expression in GEM, assayed either by enzyme activity or immunofluorescence. The most commonly and widely applicable reporter genes in engineering metabolic pathways are lacZ (Figure 10), gap (green fluorescent protein), gus, and lux [145].

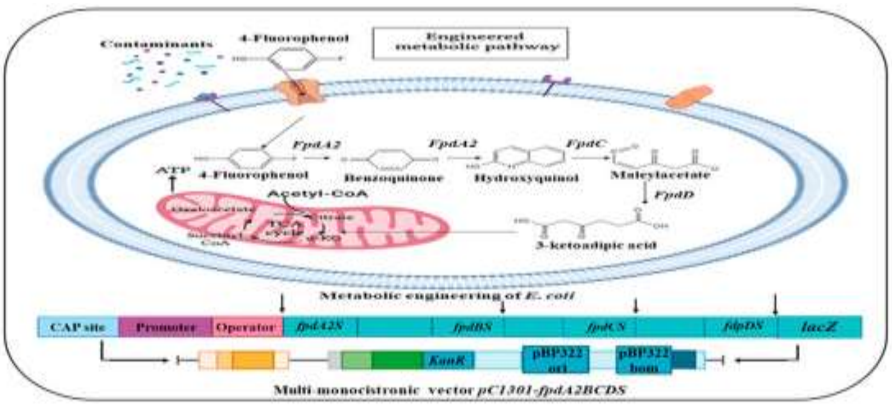

Figure 10: LacZ-engineered metabolic pathway

Regarding the competition with native species, as noted by de Lorenzo [229], the risk of altering the microbial composition by introducing GEMs into natural ecosystems is not as high as commonly perceived. Owing to the homoeostasis of biological ecosystems and resistance to colonization, engineered microorganisms have difficulties establishing in a new environment, meaning that it is difficult and unlikely that the modified microorganisms could displace the indigenous community [146, 147]. Research has shown that rate-limiting steps in known metabolic pathways can be genetically manipulated to increase degradation rates, or completely new metabolic pathways can be integrated into bacterial strains for the degradation of previously recalcitrant compounds. Other strategies using engineered microorganisms for process monitoring and control, toxicity and stress response assessment, and complete hydrocarbon cleanup are also cultivatable [137].

Application of Artificial Intelligence in Bioremediation

AI concerns itself with building machines and computers with reasoning ability that normally only average human capacity can develop or involves data whose scale supersedes human ability to analyze. The integration of artificial intelligence with biological, chemical, and biochemical processes is an active research and development aspect in this contemporary time. AI is gaining much recognition in modern times, especially in the fields of applied sciences, biomedical and medical sciences, and agriculture. Various applications, such as pattern recognition, disease diagnosis, image understanding, intelligence search, automatic programming, and human and robotic games, are influencing man and his environment greatly [148].

An assemblage of biological systems with the potential of algorithms to broaden the scope of bioengineering is exciting [149]. Numerous AI tools have been applied in the analysis of biochemical processes, of which, artificial neural network (ANN), Monte Carlo simulation (MCS), immune algorithms (IA), boosted regression tree (BRT), and ant colony algorithm (ACA) have been duly applied by researchers. The use of artificial intelligence (AI) in environmental quality assessment and monitoring is becoming a field of interest as researchers become curious about devising new techniques aimed at obtaining more accurate and precise results in less time. Modern machine learning methods aid in interpreting high-dimensional and nonlinear data [150]. In the investigation of Kumar and Mathur [151], an ANN was used instead of a BIOPUME III to stimulate the biological degradation of HCs. However, they employed a procurator model in constructing the ANN to optimize the process using the Levenberg–Marquardt back propagation algorithm.

The engagement of established mathematical and statistical models in parameter optimization plays an important role in environmental degradation by biological means. Bordoloi et al. [152] employed RSM to optimize various growth conditions affecting Achromobacter sp. bacterium isolated from contaminated petroleum soil in examining the desulfurization of dibenzothiophene (DBT), which is preponderantly concentrated in diesel. Similarly, Ramasamy et al. [153] employed RSM growth condition optimization for the culturing of Enterobacter cloacae (KU923381) in degrading diesel. Although it is evident that statistical modeling and conventional mathematical algorithms have long been incorporated in the study of biochemical processes such as bioremediation, only a few studies have employed AI in process description and monitoring. However, AI can efficiently assist in monitoring environmental contamination sites. Jiao et al. [154] designed a novel technique for the automated detection of oil spills that are difficult to detect by employing three units: crewless aerial vehicles (UAVs), a conventional algorithm, and deep learning, operating independently to achieve the task. First, a deep convolutional neural network-based model capable of detecting oil spills as images was designed, assuring no exclusions. Second, an Otsu algorithm was used to increase the precision of the detection task to eradicate other errors or noise in the seen images. Finally, a maximally stable extremal region algorithm (MSERA) was used to secure the polygon from the detection/sensing box. The design was not only successful in detecting oil spill areas but also helped in reducing the cost incurred in oil spill detections by 57.2% when compared to the conventional detection methods. Sanusi et al. [155] conducted a comparative study to optimize TPH degradation in diesel-contaminated soil by Paspalum scrobiculatum, a tropical plant, using RSM and ANN. An optimum condition was attained at a diesel concentration of 3% with 72 days of sampling, and 1.77-mL/min aeration in the case of RSM, leading to 76.8% TPH removal, whereas, in the case of ANN, the predicted optimum condition was at a diesel concentration of 3% with 72 days of sampling, and 1.02-mL/min aeration, in which 85.5% TPH was removed. Thus, the ANN is more accurate than the conventional RSM model in data estimation and fitting. Some researchers have studied the adsorption and diffusion of 16 PAHs in silica nano-pores, laying key emphasis on: free surface area, mean square displacement, adsorption energy, and volume fraction while incorporating molecular dynamic simulation (MDS) [156]. Applying linear and nonlinear regression using the partial least square (PLS) method with various machine languages such as: support vector regression (SVR), M5 decision tree (M5P), and multilayer perceptrons (MLP), to obtain information about the influence of various factors on adsorption. The study concluded that PAH sorption was facilitated by the diffusion mechanism. However, the combined approach, including MD and machine languages, can aid in deciphering the segregation of organic contaminants in soil particles.

Wang et al. [157] employed an AI system termed an integrated extended short-term memory network (LSTM). By employing cross-correlation and association rules, LSTM identifies point sources of pollutants and as little as small indefinite amounts quantity of contaminants of industrial origin concentrated in water bodies. These cross-correlation maps were designed to aid in tracking point sources of contaminants. AI has been applied not only in tracking biochemical processes but also in forecasting parameters. Shadrin et al. [158] incorporated ANN and support vector machine (SVM) machine languages in predicting and estimating phytotoxicity of petroleum. In this study, 11 soil samples collected from Sakhalin islands under greenhouse conditions were analyzed using ANN and SVM to predict petroleum toxicity levels in plants. The models also helped in analyzing the soil properties, which is usually a time-consuming and operose exercise. In 2021, a scientist named Dragoi, along with a team of other research colleagues, utilized a neuro-evolutive methodology involving ANN and DE (differential evolution) to predict TPH and OC (organic carbon) concentrations, two key factors of oily sludge composting that determine the efficiency of petroleum removal process.

In interpreting petroleum rheology, AI has played a key role, especially in the aspect of crude oil mobility. Stratiev et al. [159] used an ANN-based model to predict petroleum viscosity. Extensive studies have continued to backbone claims and affirmations of the panoptic application of AI and its importance in enhancing the accuracy of petroleum pollution assessment and monitoring in affected regions. Therefore, the combination of artificial brains (i.e., machine learning algorithms) with conventional remediation technology improves the restoration process, making it more precise, faster, more cost-effective and time efficient – presenting a means to predict the possible extent and time span of a remediation process. The trend of AI-based remediation studies has been tremendously escalating in recent times. Furthermore, applying computational and statistical techniques to biological data processing and analysis advanced bio-informatics tools (particularly in the space of genomics) is adaptable in examining the decomposition patterns of the hydrocarbon compounds and in disambiguating catabolic pathways adopted by the hydrocarbonoclastic microbes in polluted environments as well as in enzyme reengineering (Figure 11 below).

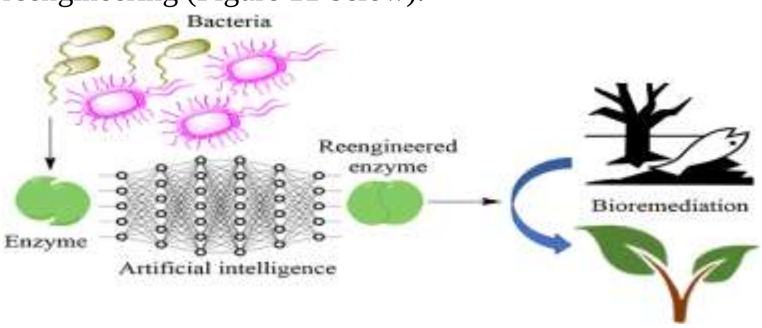

Figure 11: Artificial intelligence in enzyme reengineering for bioremediation application

Hopefully, a database can be developed in the future that would contain detailed information on different petroleum-polluted sites, capable of giving researchers and environmentalists insight about an area polluted by petroleum and possibly other bio-remediable pollutants. However, it is anticipated that research studies on AI integration in bioremediation will find enormous importance in solving environmental issues.

Biosensing

Because of the harmful impact of environmental petroleum contamination on living organisms, the importance of risk assessment cannot be overemphasized [160, 161]. In view of containing and mitigating environmental pollution in the shortest achievable time span, the development of a detection unit for these pollutants is a prerequisite [161, 162, 163]. Challenges arising because of implementation cost, time requirement, and the complexity of procedures must be addressed. Novel and cultivatable techniques characterized by high degree of efficiency, sensitivity, and rapid detection must be developed [160, 163]. A biosensor is an integral and analytical device that can detect changes, modifications, and mutations as signals in biochemical processes, providing them as data for quantitative and qualitative analysis/interpretation of the process. Biosensors comprise three main components: a biological recognition element (receptor), a transducer, and a signal processing system (detector) [164, 165, 166]. The receptor can be enzymes, antibodies, antigens, nucleic acids, or even whole cells. The detected biochemical signals could be derived from metabolic processes, gene expression, cellular toxicity, or enzyme activity. Finally, the transducer can be classified according to its physicochemical nature to detect electrochemical, optical, calorimetric, or thermal signals [165, 166]. A liaison of the biological sample with the receptor generates a code that is converted by the transducer into a quantifiable electrical signal [161, 164, 165, 166]. This means that the biological recognition element selectively identifies the analyte, i.e., the substance whose chemical constituents are being measured, by generating a specific signal. An analyte in a bioremediation process is a contaminant. However, for an analyte to be identifiable, it must possess a special group detectable and distinguished from others by the transducer. The type of signal generated depends on the type of transducer used. Therefore, the resulting quantified signal depends on the nature of the transducer employed [161]. Biological systems may comprise multiple analytes that require biorecognition elements capable of monitoring diverse toxic components. However, antibodies are one such element capable of simultaneously and selectively monitoring multiple samples with a fast response [161]. In the petroleum industry, environmental monitoring of bioremediation processes is of paramount importance to ensure the safety of the adopted procedures, reduce possible future contaminations [167, 168], and prevent stale pollutants from becoming xenobiotic in the future. In so doing, the potential risks can be eliminated by ensured timeliness in detecting toxic compounds [162]. There are physicochemical methods that evaluate oil contamination [90]. Although effective, they are characterized by high implementation costs, time consuming, laborious, and may require a large sample volume for analysis [160, 167]. Molecular tools offer an alternative approach to surmounting these challenges [167]. They can also be cultivated on a large scale and even be engineered to resist harsh conditions, such as extreme pH and temperatures, and environmental contamination [164, 165]. Biosensing can be optimized using synthetic biology. With the possibility of constructing whole-cell biosensors (WCBs). In monitoring and tracking environmental pollutions of heavy metals, pesticides, pharmaceutical residues, chemicals, and organic pollutants of petroleum origin [169], WCBs have been used as a result of their diverse baroreceptors that can be used along with different genetic mechanisms to overcome traditional sensors [167]. The use of microorganisms to monitor different pollutants is a result of the development of genetic engineering techniques inculcated with biosystem, which have made it possible to modify or design the necessary elements for signal detection and processing [170].

The premier genetically modified microorganism to be used as a WCB for the monitoring of the bioremediation process of contaminated soil was Pseudomonas fluorescens (HK44 strain), which contained the pUTK21 plasmid. This plasmid contains the nahG gene (which controls salicylate degradation) fused with the LuxCDABE gene cluster [171]. Patel et al. [172] developed two biosensing strains to detect hydrocarbons. Two vectors were designed with a promoter-operator fused with fluorescent protein genes: tbuT-gfp (capable of detecting BTEX - benzene, toluene, ethylbenzene, and xylenes compounds) and phnR-cfp (capable of detecting naphthalene, phenanthrene, and related PAH compounds). The designed vectors were then transfused into E. coli DH5α. Both recombinant strains were capable of detecting monoaromatic and polyaromatic hydrocarbons, thereby creating a method for measuring contaminant levels [172]. Roy et al. [173] combined synthetic biology with a complementary design to construct a genetically rewired and selective biosensor, which is ideal for detecting pollutants in contaminated water sources [173]. This study aimed to create an adaptable WCB that can be refined to suit a particular mono-aromatic contaminant. This study based its design on the phenol catabolism pathway, using the MopR protein to trigger the transcription of the gene cluster when binding to phenol. In this case, the difference was that the catabolism gene cluster was replaced with a reporter gene (Luc gene) to achieve sensitive detection, creating the MopRLuc biosensor, capable of detecting low and high concentrations of phenol in water. Given the efficiency of the constructed biosensor, Roy et al. [173] decided to use it as a model to create an array of WCBs for other pollutants. They discovered that mutations in sensor profile were due to changes in the variable region, allowing the generation of biosensors capable of detecting xylenol, ethylbenzene, and xylene. Not only did this study obtain successful results, but it also created a biosensor template that can be manipulated for the detection of various aromatic pollutants in the environment [173]. Some areas in biosensor development still need to be studied to overcome limitations, such as sample interferences, cell stability, hydrocarbon bioavailability, and even legal considerations on the release of engineered microorganisms into nature [167, 168]. However, synthetic biology brought new possibilities in the creation of biosensors to help achieve early detection of toxic compounds [162].

Nanotechnology in nanobioremediation

The principles of nanotechnology have recently been widely exploited and successfully applied in various fields. In environmental sciences, nanotechnology has been duly applied in accelerating entoxicant transformation to improve the activity and effectiveness of conventional methods such as biological remediation of contaminated regions [174].

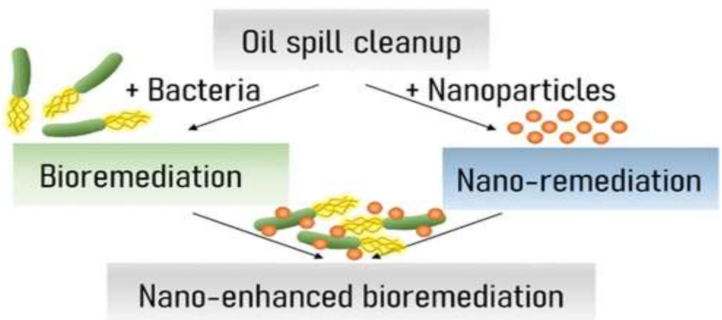

Figure 12: Nano-Enhanced Bioremediation for Oil Spill Cleanup

Biological processes have been improved through the integration of biotechnology and nanotechnology principles (also referred to as bionanotechnology/nanobiotechnology by some authors) (Figure 12). Nanotechnology and biotechnology principles can be applied collaboratively in bioremediation to help nurture the environment by altering its characteristics and consequently enhancing the contaminant removal rate and efficiency [175, 176]. Nanotechnology is a multifaceted technology that can play a vital role in the bio-stimulation of a bioremediation process. Approximately 10 million tons of toxic chemical compounds are released annually by the industry [177, 178, 179, 180]. When liberated into the environment, these compounds might further react to form complex chemicals that are even more toxic than their parent compounds. The wide variability in the physicochemistry of such chemical compounds, their cytotoxicity level, and their multiple interactions with biotic and abiotic environmental factors (viz., microorganisms, plants, animals, water, minerals, organic matter, wind, etc.) may complicate the implementation of simple remediation techniques [181, 182, 183]. The combined use of nanomaterials (NMs) and nanoparticles (NPs) with biotechnologies has the potential of offering a step-change in remediation capabilities, avoiding process intermediates, and advancing degradation speed [184, 185]. Nanoremediation technology is a sustainable method to reduce soil contaminants. The use of nanoparticles in enhancing the microbial activity of degraders is one aspect of nanotechnology that is yet to be explored by many but a few researchers in the world. Bio-stimulants with smaller particulate sizes owing to greater surface area could serve as better arousers and augmenters (for cases where they serve both purpose of augmenting and stimulating) in the bioremediation process due to greater contact surface, and can in other words be referred to as nano-biostimulants.

Nano-biostimulants—like all NPs, have particulate sizes that fall within the nanoscale (i.e., 1 - 100nm) level. Nanotechnology has gained much attention in the present era due to its unique properties and higher efficiency [186]. Compared with bulkier and larger counter molecules, nano-molecules offer better efficiency and enhanced reactivity because of their higher surface-to-volume ratio [187]. Bioremediation is a redox reaction, and the recent use of microorganisms to synthesize functional nanoparticles has been of great interest. For example, in the bioremediation of heavy metal (HM)-polluted soils, their action can change the oxidation state of metals and sometimes with the addition of chemically synthesized metallic nanoparticles. Biochemical processes can be made eco-friendly when nanobio-fabrications are coupled with the simultaneous use of microbes. This is a sustainable means of achieving bioremediation.

Recently, NMs have been incorporated into bioprocesses with the sole aim of promoting and increasing the half-life of environmental intoxicants [188]. Cecchin et al. [189] referred to nanobioremediation as the use of NPs and living organisms (microorganisms and/or plants) in the detoxification of environmental pollutants. Furthermore, El-Ramady et al. [190] described this phenomenon according to the nature of the living organism employed in the remediation process. However, the processes were specifically termed phyto-nanoremediation for nanobioremediation involving plants, microbial nanoremediation for nanobioremediation involving microorganisms (usually bacteria), and zoo-nanoremediation for nanobioremediation involving the use of indigenous soil biota or invertebrates. The interaction between group with NP and the degrading microbes must be corrected if an optimum remediation process is required. In this context, the NP characteristics must be well designed. Characteristics (such as nanotoxicity, particulate size within the nanoscalescale, nanonutrition, etc.) can be rate determining and if optimized, will promote the degradation rate, but if not, can suppress the rate of remediation even below the natural attenuation rate.

Nanoparticles in the environment can be harmful; however, by compounding them with enzymes of microbial origin, nanomaterials can be altered and modified into less harmful materials in the environment. When enzyme molecules are present with nanomaterials, their cell interaction is minimized through steric hindrances and a decrease in the active surface energy is promoted [191], which is necessary in retrograding their harmful effects in cases where they are malign. Enzymes are ecofriendly (a green means to remediation) and provide a supplementary distinctiveness of contact action (catalysis), which makes NMs more adaptable and efficient in bioremediation [192]. Grat and Kim [193] developed a synthetic protocol for both stable and active enzyme system –"Single Enzyme Nanoparticles" (SENs). The combination of SENs and nanostructured matrices will greatly impact the involvement of biosensors (for example, in environmental monitoring) and bioconversion (for example, as in bioremediation). Although this has several advantages in bioremediation, it also has many drawbacks. Integrating nanotechnology with bioremediation can result in exceptional and extraordinary adsorption capacities as a result of surface effects, small size effects, quantum effects, and macro-quantum surface effects [194]. In general, nanobiotechnology is geared toward developing biosynthetic and ecologically friendly technology for the nanoscale synthesis of materials. The large surface area of the nanoparticles due to their small size promotes high reactivity and catalytic action. Various physicochemical factors, such as bioavailability, seem to be the rate-limiting factors of a bioremediation process. Nano-biostimulation influences bioavailability in that the increased surface area makes the substrate easy to breakdown and sorb. Because of increased bioavailability, the growth rate is promoted over decreasing substrate concentration (since the substrate has been used up, its concentration diminishes over time).

Reduced particulate size and increased surface area of bio-stimulants also promote homogeneity. These are some of the advantages of using nanobiostimulants in synergy for remediation processes. The pH of prospective nanobiostimulants can be easily altered to suit the environment and promote microbial life and sustainability. Microbial NPs are highly affected by changes in parameters such as temperature, pH, pressure, and particle size [195], which tend to be rate limiting. Although pH is a chemical characteristic of a species, particle size distribution influences material characteristic properties such as flow, reactivity, solubility, and compressibility, which greatly influence pH alteration. Brownian motion is promoted by an increased degree of particulate dissociation and consequently increased fluidization/powdering and reactivity due to increased contact. For an NM to serve as a nanobiostimulant, it must be degradable. Three major attributes important for the applicability of nanobioremediation are as follows [196]:

1. Green and clean nanomaterials are used;
2. Solution for the removal of contaminants
3. To be used as environmental agent sensors.

The improved properties of nanoparticles are one of the distinctive qualities that promote their extensive use and application in various fields of study, such as medicine and pharmaceuticals, drug delivery, and environmental protection. The zeta potential, surface chemistry, photocatalytic properties, and chemical composition define the chemical properties of nanoparticles [197]. In environmental remediation, small-sized nanoparticles are easily distributed across a greater area of surfaces and into target locations, aiding in discharging occluded and constellate water-insoluble materials, which is a necessity in aquatic environment-related bioremediation. In aquatic environments, the solubility of nano-biostimulants is a key factor to consider, and this is why the role of enzymatic biosurfactants should be studied in detail. Nano-particles synthesized by various chemical and physical methods are prone to have devastating effects on the environment and interacting microorganisms. In recent times, many researchers have emphasized the synthesis of green nanoparticles (i.e., the synthesis of nanoparticles using microorganisms or plant extracts). Biological substances are more easily degradable than their chemically synthesized counterparts. Concerning this problem, however, scientists are attempting to apply new methods to other technologies to remove nanoparticles from marine ecosystems [198]. Methodologically, pollutants can are removed by adsorption–physisorption and chemisorption, the major mechanisms through which the biogenic nanoparticles interact with pollutants to detoxify the environment. Chemisorption is the type of adsorption in which strong chemical bonds are formed between the adsorbate and the adsorbent surface; hence, new compounds are formed by redox at the adsorbent surface. On the other hand, physisorption is the type of adsorption in which the adsorbate is attached to the surface of the adsorbent with weak van der Waal’s forces of attraction; hence, no new compound is formed in the process. Comparatively, the chemical adsorption method is considered as a better method for remediation [199]. On reduction to low toxic levels, these NMs can be easily biodegraded, with a consequential increase in biodegradation rate [197]. In this way, toxicity levels are no longer rate-limiting. One of the most attractive features is the small quantities of NPs required in applying nanotechnology. This has potentially reduced the cost associated with the application in the bioremediation of petroleum-contaminated soils. However, economic analysis for up-scaling is deficient. While bench-scale progress has been made regarding nanoscience for oil spill response, more large-scale research is undoubtedly needed before these technologies can be scaled up for commercial use [26]. Tan et al. [200] noted that the physical and chemical interplays of nanoparticles, soil biota, and contaminants are dependent on a variety of parameters, including nanoparticulate size and orientation, surface coating, chemical nature of the nanoparticle and contaminant, type of organism, media, pH, and temperature. Kumari and Singh [188] demonstrated the usefulness of nano-matter in facilitating bioremediation processes considering their role in microbial growth enhancement, immobilization of remediating agents, or induced production of microbial enzymes responsible for remediation. A similar study demonstrated the role of nanomatter in facilitating biosurfactant production for improved contaminant solubility and increased bioavailability [201]. The interactions between nanomatter and biosurfactants could be modeled in channeling biostimulation; however, this technique is sometimes referred to as nanobiostimulation. Biostimulation has been able to tackle the problems of rate limiters in the bioremediation of oil spill sites; nonetheless, research has proven the potential of nano-biostimulation in efficiently amending contaminated environments using inexpensive, fast, and ecofriendly procedures. Nanotechnologies used in bioremediation processes are expected to drive the technological evolution for improving the environmental quality in developed and emerging countries [202, 203]. A significant amount of research has been performed to determine the mechanisms of decontamination and remediation [204]. Furthermore, world markets for nanotechnology and bioremediation are expected to conceptualize novel niches to improve not only environmental facets but also living standards in general [205]. In addition to their positive effect on the removal of these contaminants, NMs could interact with biotic and abiotic elements in both positive and negative ways. Therefore, conscious efforts have been made in research to evaluate the synergistic effect of the combined use of NMs and bioremediation practices in elucidating their physical, chemical, and biological interactions in the natural ecosystem [205].

Factors influencing bioremediation

Iturbe and Lopez [206] investigated bioremediation for an artificially contaminated soil and discovered that by controlling the factors that influence biodegradation, it was possible to stimulate the native/indigenous microbes to favor biodegradation of the hydrocarbons present in the soil. The oil-degrading ability of bacteria in tropical soil has been reported to depend on the adequacy of several environmental factors, such as temperature, moisture, nutrients, pH, oxygen availability, oil density, and soil texture [207, 208, 209, 210, 211], among other numerous physical, chemical, and biological factors that interact to influence oil biodegradation [212]. Bioremediation is aimed at sustaining the maximum possible growth of hydrocarbonoclastic microorganisms responsible for remediation of contaminated sites. Some physical and chemical factors influence the efficient remediation of polluted sites.

Previous research has shown that these physicochemical factors are highly influential in maintaining the maximal microbial growth rate:

i. **Water:** Water content is a highly influential factor instrumental to the recovery of polluted sites, as water constitutes 80%–90% of the weight in the molecular composition of bacterial cells and is the main nutrient [213].

ii. **Aeration/Oxygen Supply:** Oxygen is the electron acceptor most commonly used by microorganisms in degrading organic compounds in an aerobic environment. At an oxygen content below 2 mg/l in the soil, anaerobic conditions are favored [207]. Aeration is very efficient at the start of contaminated site treatment; hence, the biodegradation process is aerobic [207].

iii. **Hydrogen Potential (pH):** The pH needed for optimal microorganisms ranges from 6.5 (slightly acidic) to 7.5 (slightly basic). The intracellular pH lies within this range. The investigation of El-liethy et al. [53], on bioremediation of crude oil-contaminated soil using E. hormaechei experimental analysis showed that cell density slightly shot up by one log at pH values between 6.0 and 7.5. However, optimum cell growth was observed at pH 7.0. After 7 days, the cell density increased by 1.16 log and 2.54 logs after 30 days. Notwithstanding, at pH 8.5, the cell density slightly depreciated by 1.9 logs after 7 days and by 2.2 logs after 21 days. This to a great extent demonstrates the importance of pH levels in bioremediation. Previous biodegradation studies revealed that pH ranges between 7.0 and 7.5 favored some bacteria's oil biodegradation abilities [214, 215].

iv. **Nutrients:** Nutrients necessary in bacterial cells can be categorized into main/macromolecular nutrients and micromolecular/trace nutrients. Nutrient deficiency can limit the metabolic activities of microbes and consequently mitigate remediation progress. Bacterial cells are composed of elements such as carbon (C), nitrogen (N), hydrogen (H), and phosphorus (P) and some trace amounts of potassium (K), magnesium (Mg), iron (Fe), chlorides (Cl), calcium (Ca), and molybdenum (Mo), with carbon as the main component at about 50% concentration [216]. In the remediation of hydrocarbon-polluted soils, the mechanism of remediation allows the oil-eating degrader to use the carbon present in petroleum hydrocarbons as a carbon source. Hydrocarbon contamination is the main component and therefore serves as the substrate in the remediation process because hydrocarbons do not resist attack from microbes. Bioremediation is an aerobic process and therefore requires oxygen supply. However, oxygen is the second most abundant element (approximately 20%) in the cell and is a good electron acceptor required for new cell synthesis. Priyom Bose [218] revealed that peptone is the most suitable source of nitrogen for bacterial microbial growth as it increased the growth rate of E. hormaechei by a factor of $2.16\log_{10}$.Other nitrogen sources, such as yeast extract and ammonium nitrate (NH_4NO_3), also increased the growth rate but did not perform better than peptone. This exclusively supports the claim that the physiological makeup of oil-degrading bacteria (ODB) is different for different species of degrading bacteria. Hence, there is a possibility that a growth-enhancing nutrient deficiency (for one or more degrading species) could be growth-diminishing for another degrading species in a polluted environment consisting of numerous ODB. Jin and Fallgren [218] reported that urea preferentially enhanced biodegradation because of its high nitrogen content and peptone as a good stimulant during oil biodegradation [219]. However, other studies have indicated contradictory findings. For instance, urea negatively impacts oil biodegradation due to its high ammonia content and acid toxicity, which significantly reduces the microbial population [220]. Furthermore, NH_4NO_3 had minimal effect on microbial biomass production and biodegradation rate because nitrate ions inhibit certain microorganisms [221]. These claims are subject to the nature and type(s) of environmental contaminants and degraders.

v. **Temperature:** Based on temperature, microbes can be subdivided into phylophiles (active at temperatures < 15°C); Mesophiles (active at temperatures between 15°C – 37°C); Thermophiles (active at temperatures > 55°C). Each organism has a minimum temperature below which growth is limited, an optimal temperature at which growth is faster, and a maximal temperature beyond which no more growth occurs. Enzymatic and chemical reactions generally increase concomitantly in cells with increasing temperature. In heterotrophic bacteria, the temperature range considered optimal is between 20°C and 35°C [222]. The E. hormaechei count and activities increased with increasing temperatures within the range of 20°C–35°C for 30 days [53]. Specifically, the density of E. hormaechei increased only slightly (0.28–1.72 logs) at 20°C, 25°C, and 35 °C. However, at 30 °C, there was a significant increase of 2.46 logs during the observation period. Temperature directly alters the chemical structure of oil pollutants and the physiology and diversity of degrading microorganisms [223, 224, 225].

Factors such as low temperature, very low or very high pH values, and chemical agents such as heavy metals, halogens, organic, and oxidizing contaminants can limit the activity of microorganisms. However, Das and Chandran [138] also showed that when nutrients and oxygen are sufficiently concentrated, there is an optimal growth rate of hydrocarbonoclastic bacteria at pH 6.0–9.0 [139].

Conclusion and Recommendations

The emergence of profound technologies aimed at cutting down on the exposure, risk, and rate of pollution caused by dependence on non-renewable energy sources (such as petroleum and coal) by switching to renewable sources of energy (e.g., solar energy) is an area of progressive research in recent times. This move is counter-productive to the high rate of environmental pollution from petroleum and its by-products. Although a move toward renewable energy sources for world energy is necessary, petroleum is not only required as a source of energy but is also necessary in other industries, such as pharmaceutical, pesticide, and plastic manufacturing industries.

Hence, it is very necessary and will continually be explored, coupled with the fact that some prominent inventions will still depend on it for its active performances, making it a front burner in society until a substitute for it is synthesized or discovered. Knowing this simply highlights the importance of improved technological means toward curbing the effects of petroleum contamination, both current and forecasted. Bioremediation is a biochemical process that could be well explained by Monod's relations for biological processes. Further research studies could be conducted to compare the rate of remediation on stimulated and unstimulated soils using the Monod's relation, inculcating AI, genetic engineering, and nanotechnological principles. In an experimental study, this could serve to provide a predictive model relating the concentration of biostimulants and pollutants with the size of the microbial community responsible for remediation.

Similar to most bond weakening and breaking chemical processes, it is endothermic and may be promoted at suitable temperature ranges. However, being a biochemical process, the activity of microorganisms cannot be neglected as they can be limited by temperature. Therefore, there is a need for an optimum temperature that is suitable and promotes microbial growth, enzymatic action, and biodegradation enhancement so as not to make temperature a rate-limiting factor in the degradation process. Therefore, there is a need for an in-depth study into the activity of different hydrocarbonoclastic bacteria relative to operating temperature. This could serve as a database to provide knowledge to researchers who seek to combine different bacteria in an experimental work. Ofeogbu et al. [2] pointed out that the bioremediation process was enhanced more in loamy-sand petroleum-polluted soil than in clay petroleum-polluted soil when stimulated with livestock waste. This could be a result of the soil's nature, such as porosity, moisture-retaining capacity, texture, and pH. However, further studies could be conducted to ascertain the rate-limiting factor in clay soils as not all polluted sites happen to be loamy-sand soiled.

One problem that some researchers tend to misplace or fail to point out is clarifying the uncertainty of knowing the precise proportion of biostimulants needed to take the remediation process from start to finish and their effects on the microbial community. This could give an idea of how much biostimulant is necessary for a remediation process to avoid the possibility of overloading the polluted soil. Although the remediation process cannot attain 100% removal efficiency [226] (i.e., there will be a residual or irreducible amount of contaminant left in the soil), the enhancement of some biotic and abiotic factors will encourage a high mitigation level, and the life of bacteria will thrive, and the residual PHC concentration in soils will be drastically reduced when bio-stimulated compared to natural attenuating soils. Genetically stronger and better modified degraders with enhanced degrading efficiency and capabilities could be engineered from known degrading ancestor organisms through genetic splicing. In bioaugmentation, added organisms may be cultured after isolation from a previous site or genetically modified [227, 228] to increase biodegradation by inducing bioemulsification [229, 230]. When choosing an appropriate bioremediation technique, it is important to know the contamination site conditions and the type of pollutant involved. However, data insufficiency can promote setbacks in the elimination process or even affect the establishment of necessary control measures. Therefore, the correct detection of contaminants is of great importance. Given that many of them can tolerate high concentrations of contaminants, have a high chance of survival, and can quickly adapt to harsh conditions, they are perfect candidates for monitoring using microorganisms. Considering that many hydrocarbons are extremely cytotoxic, synthetic biology creates the possibility of engineering microorganisms with the necessary machinery to not only detect different pollutants but also possess characteristics that inhibit their growth. Studies conducted in such areas provide data to position biosensors as new effective systems for detecting hydrocarbons in affected environments. The development of biocompatible nanoparticles for bioremediation can be effective in the treatment and eradication of petroleum and other similar pollutants in the environment. Special attention and focus should be given to green nanoparticles synthesis over physically- and chemically-synthesized nanobiostimulants to prevent or curb the possibility of the emergence of further environmental problems in the future. Cost-effectiveness and efficiency are very important factors in the design of an engineering process and should be well emphasized. Nanoparticles sourcing and procurement should be economically feasible for large-scale applications in the restoration of contaminated sites. The extent of a bioremediation process can be foretoled by the growth kinetics study. In addition, the mass balance and stoichiometry of the hydrocarbon contaminants subjected to bioremediation are essential in understanding the conversion process and estimating the effectiveness of the process. Bioremediation process biostimulation should aim not only at soil remediation but also at the development of an effective waste management technique by employing degradable environmental wastes (as bio-stimulants) to excite the process. Some degradable wastes are common to the suburbs of Africa. The use of common agricultural wastes is a cheap, potent, and effective method that has been shown through research over the years compared with the use of synthesized nutrients, such as agar and NPK fertilizers, which are inefficient in terms of cost, especially if remediation is to be carried out in an up-scaled process or at the field level. Zhang et al, 2020 [95] reported that an increase in nutrient load due to pollution could increase the total environmental microbial population, but this claim is subject to the degree to which other environmental factors, such as the degree of toxicity, affect the microbial community. However, care must be taken to avoid the possibility of soil overloading. The feasibility study and acceptability of an approach or technique aimed toward effective remediation is subject to factors such as cost management efficiency, and more so ecological and human compatibility/effect and not only based on the efficiency recorded from its adoption and practice. Although the dependence on petroleum as an energy source is great and its versatility cannot be discarded, the effect of its pollution in the environment has informed the world of the need for a switch toward cleaner and renewable sources of energy.

Finally, the dynamism leading to advancements in scientific and technological approaches borne from an ever increasing call for and implementation of research findings in environmental remediation, the idea of incorporating AI, nanotechnology, genetic engineering, and other advanced techniques in bioremediation (and other biochemical processes in general), is a commendable concept toward discovering novel, economical, and enhanced techniques aimed at efficiently and cost-effectively mitigating environmental contaminations in general. However, the complexity of such systems may be challenging.

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