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Bioremediation of Crude oil-contaminated Soils – *A Review*

Ifeanyi Michael Smarte Anekwe, Yusuf Makarfi Isa

School of of Chemical and Metallurgical Engineering, University of the Witwatersrand, Johannesburg 2050, South Africa

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Abstract

Pollution has contributed immensely to the degradation of the environment which posed a prolific threat to the availability of potable water and land. Polluted sites are now widely regarded as a potential threat to human health, and the growing discovery of polluted sites in recent years has triggered global efforts to effectively address many of these sites, either to avoid serious health or environmental consequences from contamination or to enable the area to be restored for use. The use of conventional, thermal, chemical, physicochemical and encapsulation methods for the treatment of contaminated sites have some rooms for improvement to meet the remediation purpose. Bioremediation offers the use of microbes to destroy, remove or minimize the concentration of toxic substances on a polluted site. This biological treatment approach can be applied to clean up polluted areas like water, soils, sludges, and waste streams, among other things using cost-effective, low-technology and environmentally friendly methods for the remediation of contaminated sites and can also be carried out on-site. This paper reviews the treatment of crude oil-polluted soils by the application of different bioremediation technologies, factors affecting the performance of bioremediation, challenges, and prospects for a successful application of bioremediation technology for the treatment of polluted sites. Future research should investigate the acceptability and integration of genetically modified microbes (GEM) using molecular technology allowing for the delineation of microbial structure and the prediction of the remediation performance of microorganisms under in-situ environment for an effective clean-up of a contaminated site.

Keywords: *Bioremediation; Bioaugmentation; Biostimulation; Bioventing; Crude oil.*

1. Introduction

The increased industrialization, globalization, and development have contributed immensely to economic growth, provision of basic human needs which makes life and task easier, but these activities do not come without its negative effect as it has continued to generate an immeasurable amount of pollution which contaminate the natural environment worldwide. Pollution is a plague threatening the existence of the ecosystem and if not properly addressed can gradually lead to the destruction of the terrestrial and aquatic habitats. These can be attributed to the activities of heavy industries such as mining operations, exploration, processing, and movement of crude oil and petroleum products [1-2].

It is indisputable that most industrial activities, (mining, oil exploration and drilling of mineral resources) contribute to the economic growth, development and sustainability of most countries that depend on mineral resources as a source of their major export earnings. Since these countries are involved in the production and processing of crude oil and the utilization of petroleum products, they are invariably prone to pollution associated with crude oil. These activities have brought more wealth to individuals and government but at the price of extensive ecological damage because of little or no strategy to effectively manage the direct and indirect effect of crude oil spillage. As crude oil production and processing operations are critical to development, hence, devising means to mitigate these contaminants and their aftermath is necessary.

Crude oil pollution can be attributed to the oil spill caused by accidents involving tankers, barges, pipelines, refineries, drilling rigs and storage facilities and can also be caused by human as a result of carelessness in equipment handling or equipment breakdown. These spills tend to pollute the natural soil environment (Fig. 1), causing variation in soil structural setting, reducing the organic content resulting in a loss in soil nutrients and low productivity due to infertility [3-4]. The presence of these pollutants in the soil reduces the availability of arable agricultural land for crop production which if not addressed might lead to low production, food shortage/insecurity and reduce exportation due to a reduction in cash crop production which will invariably harm the country's economy.

The widespread of this pollution, following the immense economic contributions, has prompted the development of several strategies and technologies required for the mitigation and/or remediation, to enhance the reclamation of contaminated sites. These methods that have been adopted so far for the treatment of crude oil contaminated sites include a physical or conventional method, chemical method, thermal method, encapsulation, soil vapour extraction (SVE). The use of these methods has been considered viable over the past years for the treatment and reclamation of contaminated sites, although some are unable to meet the remediation objectives, hence, not sufficient [5].

Figure 1. Crude oil-polluted sites *(Source; Environmental Pollution Centre)*

1.1. Effects of crude oil contaminants on the environment/soil and plant growth

Crude oil is a complex liquid comprising thousands of hydrocarbon components and heavy metals. It is precarious to evaluate the exact level of toxicity of complex mixtures when deposited in the soil especially when there's little or insufficient information to prior addition or deposit of such mixture in the soil [6]. The existence of crude oil contaminants in the soil changes soil characteristics. Soil pH and TPH content increase with an increase in the amount of hydrocarbon in the soil which causes altercation in the native soil environment, while N & P content decreases with a corresponding increase in the soil toxicity resulting to decline and loss in the availability of essential nutrients required for plant growth [7]. The high concentration of the crude oil contaminant contributes to the deterioration of soil structure and fertility which renders the soil impotent for agricultural purposes and reduces the availability of arable lands.

In a study to access the impact of crude oil on the growth, germination and grain yield of maize, *Zea mays* observed a reduction by 95% in comparison with the control experiment as reported by Ekundayo *et al.* [7]. The study recorded that oil contamination had a negative impact on maize growth parameters (plant height, leaf area, stem girth, ear length) that were investigated. The prompt germination of unpolluted samples, when compared to the polluted sample, justified the negative impact of oil contaminants in the soil and plant growth. The delay in germination was due to the absorption of oil by seeds and its penetration into the embryo [6]. Odu [8] observed that the penetration of soil by crude oil spillage causes a visible nutrient deficiency in plants, insufficient aeration, a reduction in the level of available nutrients and a significant rise in the toxicity of elements such as Mn and Fe [7] where the insufficiency of nutrients corresponds to low water intake by plants [9] which accounts to the poor performance of crops planted in the oil-polluted environment. Similarly, the effect of oil in the germination of *Zea mays* was investigated [10] with seedlings planted in the soil of different concentration (0 to 10.6% w/w) of crude oil for six weeks recorded that the growth, germination, and yield drastically decreases as the concentration of crude oil increases. At 4.2% (w/w) concentration, the average reduction was reported at 50% and 92% for germination and yield respectively. The poor growth of *Zay mays* was attributed to the suffocation of the microbial and enzymatic activities of the plant due to lack of air caused by exhaustion of oxygen by increased microbial activities.

The study by Abosede [11] to evaluate the impact of crude oil contamination on some soil characteristics using samples from contaminated site and control (unpolluted) at three different soil depths, recorded that crude oil has no significant effect on particle soil sizes (silt and clay) at different depths, however, the depth of sand particle were higher at 0.5cm depth than 10-15cm by 43.35%. The study reported that crude oil has no significant effect at different depths, but can be noted that the presence of the pollutant increases the bulk density while reducing the total porosity of the pore spaces attributed to clogging or blockage of pores spaces with crude oil, limiting drastically air and water circulation within the natural soil environment [11]. It can be suggested according to Abosede [11] that physical soil properties like saturated hydraulic conductivity, macro-porosity and total porosity and bulk density can be affected since these properties are controlled by pore spaces present in the soil.

The study by Baek *et al.* [6] to ascertain the extent of inhibition of oil contaminant on the growth of corn (*Zea Mays*) and Red bean (*Phaseolus Nipponese*) using 300g of soil artificially contaminated with varying degrees (0, 1, 3, 5, 10% w/w) of crude oil and 5 seeds each of corn and red bean was planted in darkness at 23^{0}C for 14 days, showed that uncontaminated soil has the highest shoot and root length which decreases in this progression: 0 > 1 > 3 > 5 > 10% (w/w). Moreover, unlike the *Zea mays,* corn finds it difficult to germinate in 5% oil-polluted soil which suggest its increased susceptibility than a red bean, as little as 1% concentration of oil reduced the root development of corn by 52% against 28% recorded for red bean while shoot development declined by 28.70% and 10.90% for corn and red bean respectively which is in contrast with the unpolluted sample without root and shoot decrease. The discrepancies in crude oil tolerance recorded by these seedlings may be attributed to the individual systematic accumulation of oil compound, availability of nutrients and cell wall structural discrepancies exhibited by these seedlings [6].

However, Agbogidi *et al.* [12] reported a significant decline in the plant height, stem and leaf diameter on the 10.4ml concentration of crude oil unlike the 5.2mL concentration with little appreciable growth. Maize varieties died in 42mL crude oil concentration after 2 to 42 hours. Also, *Zea mays* var. F27 (corn) was reported [13] to be destroyed in the presence of crude oil at 31ml. To elucidate the above, Ogboghodo, Erebor [14] noted that plant height, survival rate, performance rate, and dry matter yield reduced with an increase in oil pollution. In contrast, Agbogidi *et al.* [12] inferred that little or small amount of mineral oil in soil has little effect (not too harmful) to plant growth but may be beneficial depending on the oil concentration and variety of the plant.

Conclusively, plants respond differently to different pollutants and toxicity due to their genetic modification and response of plant systems as modified by environmental influences and sub-lethal effect can be linked to the presence of metallic ions and trace metals in crude oil polluted soil [7].

2. Treatment of soil contaminants

Various treatment methods have been adopted previously for the reclamation of polluted soils. The ***Physical or conventional method*** involves the digging-up of the polluted soil and taking it to a dumpsite as a form of disposal. This method is tedious, requires enough equipment, manpower, and transportation of the hazardous material from one place to another might be risky and non-eco-friendly. This approach transferred the contaminated soil from one place to another, without the application of any further treatment to the soil. Hence, the problem is temporarily solved by making the site available for use while the soil remains untreated, thereby resulting in pollution of the new landfill site.

The ***Chemical method*** employs selected chemicals for the reduction of contaminant concentration in the soil and makes it less toxic, harmless to the water bodies, soil, and human beings. The application of the chemical method of soil remediation is limited by some factors which include soil texture, type of contaminant and metals present, organic matter, and concentration of the chemical selected for the remediation process. However, harmful effects and concentration of the chemical materials (like metallic oxides) are to be considered during the pilot and large-scale applications. Some chemical remediation methods include chemical fixation, chemical oxidation, chemical leaching, and electric kinetic remediation [5].

The ***Thermal method*** involves the use of heat/heat injection into the contaminated soil to facilitate the removal of wastes or constituents of the contaminants with low boiling point by converting them into vapour, which can be collected and treated in a gas treatment facility. Sequel to the broad application of this method on contaminated site, the high energy requirement can be detrimental to the soil properties which might complicate the whole remediation process [15]. The lack of universally accepted application procedure/technology for thermal remediation, due to insufficient work to improve environmental sustainability, suitability, and applicability of this remediation method results in varying considerations for specific contaminants, site, and soil type. Hence, the choice of application is solely a factor of the contaminated soil category, heat requirement of the soil, soil features and nature of the area [15].

The ***Encapsulation method*** of soil remediation requires filtration of contaminants from the soil. The most common type of encapsulation is mixing the contaminated soil with lime, cement, and concrete, to prevent other soils from direct contact with the polluted soil. With this method of soil remediation, restoration of soil organic matters is not guaranteed as the additives might cause further deterioration of soil properties and organic content even though the removal of the contaminant is achieved. The encapsulation method of soil remediation proves to be potent in the reduction of the concentration of heavy metals constituents of the contaminants present in the soil, by precipitation and pH increase [16]. This method is considered if the soil will not be used for future purposes especially for cultivation and agricultural purposes since a reduction in the soil fertility and organic content is prevalent after the application of this remediation method. These methods are less reliable and sustainable for the remediation of polluted soil due to the high cost of operation, risk of recontamination of site, inability to ensure restoration of soil fertility and the non-eco-friendly nature of these approaches.

2.1. Bioremediation strategies

The inability of the previous treatment methods of contaminated soils to effectively remediate the soil and reduce the concentration of pollutants without posing environmental problems prompted the emergence and development of bioremediation technology. Bioremediation is an approach that offers the use of several biologically induced methods in the remediation of polluted soils. Bioremediation utilizes the activities of microorganisms to disintegrate biodegradable pollutants in the presence of favourable site parameters for effective biodegradation and performance [17]. Bioremediation provides green, safe and reliable technology for pollutant clean-up, removal, de-pollution, remediation and reclamation of contaminated sites by microbial activities. This technique has proven to be the most sustainable measure for the remediation of polluted soils and water to restore its original state [18]. In this study, remediation is defined as the act of gradual degradation, reduction, conversion or/and metamorphosis of pollutants into less harmless innocuous material by stimulating the rate of microbial activities.

In the course of biodegradation, the contaminants are disintegrated into smaller molecules by pollutant degrading microorganisms [19]. This mechanism of bioremediation involves the limitation of transfer or movement of contaminants from the contaminated area to other areas within the soil or water environment. Restriction of the mobility of pollutants enhances concentration within a specific area, improve the bioavailability of contaminants to the pollutant degrading microbes, to promote the biodegradation efficiency of the contaminant without posing an environmental risk. This process ensures the feasibility and reliability of the bioremediation approach. Bioremediation offers a sustainable, reliable, low cost and eco-friendly approach for the treatment of pollutants (e.g. crude oil) within terrestrial and aquatic habitats through the application of indigenous microorganisms or microbial consortium for the catalytic process [18]. There are different bioremediation approaches used in the treatment of polluted soils and water as shown in Figure 2. The treatment of contaminants using at the same site contamination is termed in-situ bioremediation while in ex-situ bioremediation contaminants can be treated somewhere outside the original site. In-situ bioremediation can be either be engineered through bioattenuation, biostimulation, bioaugmentation, bioventing and phytoremediation technology or intrinsic bioremediation in which the inherent abilities of naturally occurring microbes are managed and enhanced without using engineering techniques to enhance the degrading process. Ex-situ bioremediation can be in the slurry or solid phase which involves excavation and placement of contaminated soil in piles to stimulate microbial growth through ventilation of the piles, (including land farming, biofilter compositing and biopiling) while the slurry phase involves the mixture of contaminated with nutrients, water and oxygen in a bioreactor to establish a conducive environment for microbial growth and to enhance remediation.

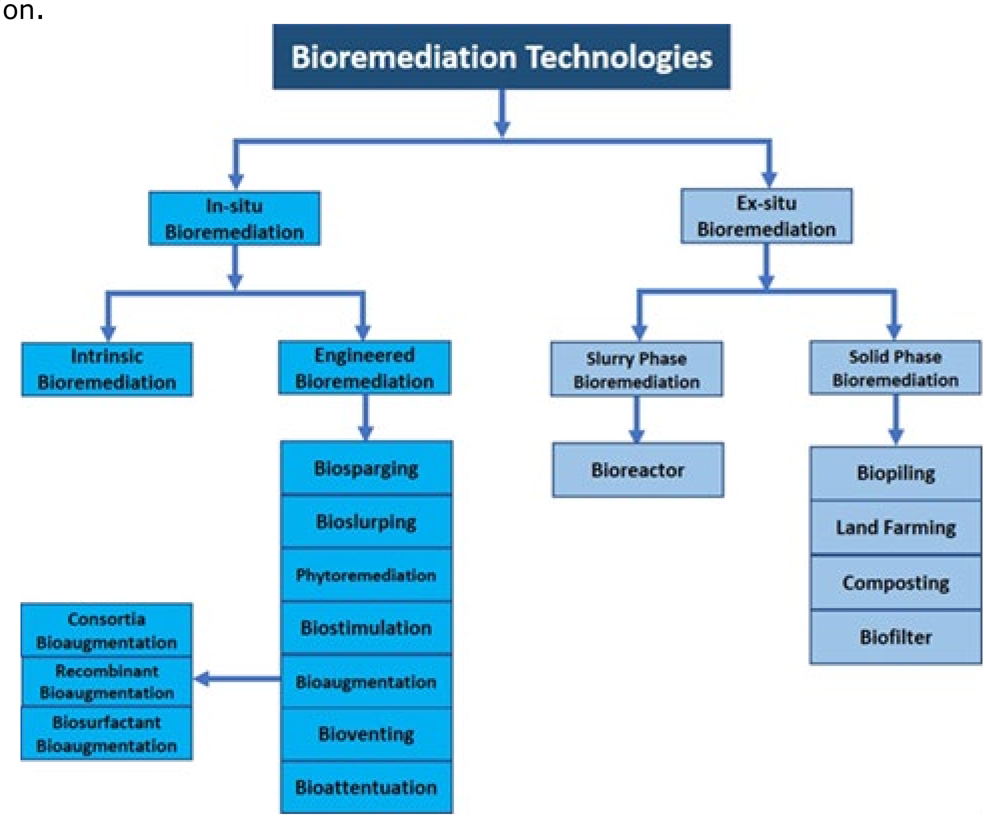

Figure 2. Bioremediation strategies for the treatment of contaminated sites *(Adapted from Sharma [20] with modifications)*

Bioattenuation or natural attenuation is a bioremediation method where degradation occurs due to natural processes without the addition of substrates or stimulants. With this method, the indigenous contaminant degrading microorganisms are allowed to degrade the pollutants in a controlled environment and prevent spread to the uncontaminated site or zone. The monitored or controlled bioremediation as an in-situ treatment method is becoming more prevalent because of its cost-effective and easy application nature but the major setback with this approach remains the slow rate of biodegradation which results in poor efficiency.

Biostimulation is a bioremediation technique where stimulants such as nutrients and/or substrates (nitrogen, phosphorus, carbon and other electron acceptors) are supplemented or introduced in treated or raw form to the polluted environment to speed up the degradation activity. In this approach, organic or inorganic nutrients are used to stimulate the natural soil environment, to support and energize the indigenous microorganism for optimum performance. The use of this method involves modification of the contaminated site using substrates to alter the existing environmental conditions, by providing a conducive atmosphere for the growth and activities of microorganisms which increases the rate of biodegradation [19]. The nutrients act as soil conditioners that facilitate and improves the soil pH, increases the bioavailability of contaminants to the degrading bacteria, promote aeration, permeability, and dehydrogenases [21].

Bioaugmentation entails the introduction of a genetically modified strain or consortium (mixed culture) of microorganisms into the contaminated environment to support, augment, enhance and improve the activities of the indigenous microorganisms for effective biodegradation. This approach is considered when the rate of biodegradation is slow due to either an insufficient or a low number of native microbes present in the contaminated site. For the successful application of this approach, selection of strain or consortium (such as oil-degrading or sulfate-reducing or heavy metal resistant bacteria) to be introduced to augment the existing organisms should be dependent on some factors like the ability of the strain to maintain stability genetically, compete for nutrient with the indigenous microorganism, tolerate high concentration of contaminants, exist in a hostile/unfavourable environment and speed up the rate of biodegradation [17]. Bioaugmentation can be achieved through the application of two or more bacterial or microbial group (consortia) or genetically modified organisms (recombinant) or biosurfactants.

Bioventing is the bioremediation process of ventilating the in-situ contaminated environment by injecting air or oxygen through the subsurface unsaturated zone to promote the dehydrogenase of indigenous microorganisms and enhance aerobic biodegradation. Bioventing aerates the vadose zone by supplying enough air/oxygen using a low flow rate to the unsaturated region for microbial activities. Air or oxygen can directly or indirectly supply into the vadose zone to enhance aerobic processes, through sparging groundwater with air or oxygen or adding hydrogen peroxide to pumped or reinjected groundwater. To enhance the efficiency of bioventing treatment, amendments are made by the introduction of nutrients (organic or inorganic) and moisture. As a result, contaminants will be microbially transformed into a harmless condition. Among various in-situ bioremediation approaches, bioventing has acquired prominence.

Phytoremediation is a method of bioremediation that employs selected plant types to eliminate, convert, maintain and/or kill pollutants in soil and groundwater. There are several distinct types of pathways for phytoremediation such as Rhizosphere biodegradation, Phyto-stabilization, photo-degradation, and Phyto-accumulation etc. Phytoremediation operates by using plants to immobilize or remove toxins from a polluted environment. The plants also convert toxic substances into less harmful substances. Some plants disintegrate organic pollutants by releasing toxin degrading enzymes into the soil or by removing and degrading soil pollutants within their tissues. In other situations, plants promote degradation by supplying the soil microorganisms with nutrients that do the job. Metals and metalloid pollutants cannot be broken down but by modifying their valence and storing them in roots or leaf tissues, plants can make them less toxic [22].

Biosparging is an in-situ remediation method that employs native microbes to breakdown organic components in the saturated zone. Biosparging involves injecting air (or oxygen) and nutrients (if needed) into the saturated zone to boost the metabolic activities of the local microbes. Unlike the bioventing that is restricted to biodegradation of contaminants in the unsaturated zone, biosparging extends the treatment to the saturated zone of the soil (Figure 3). The adaption and implementation of vacuum-assisted dewatering technique to rehabilitate hydrocarbon-contaminated soils is known as bioslurping. Bioslurping combines features of bioventing and free product recovery to deal with two different types of contaminants.

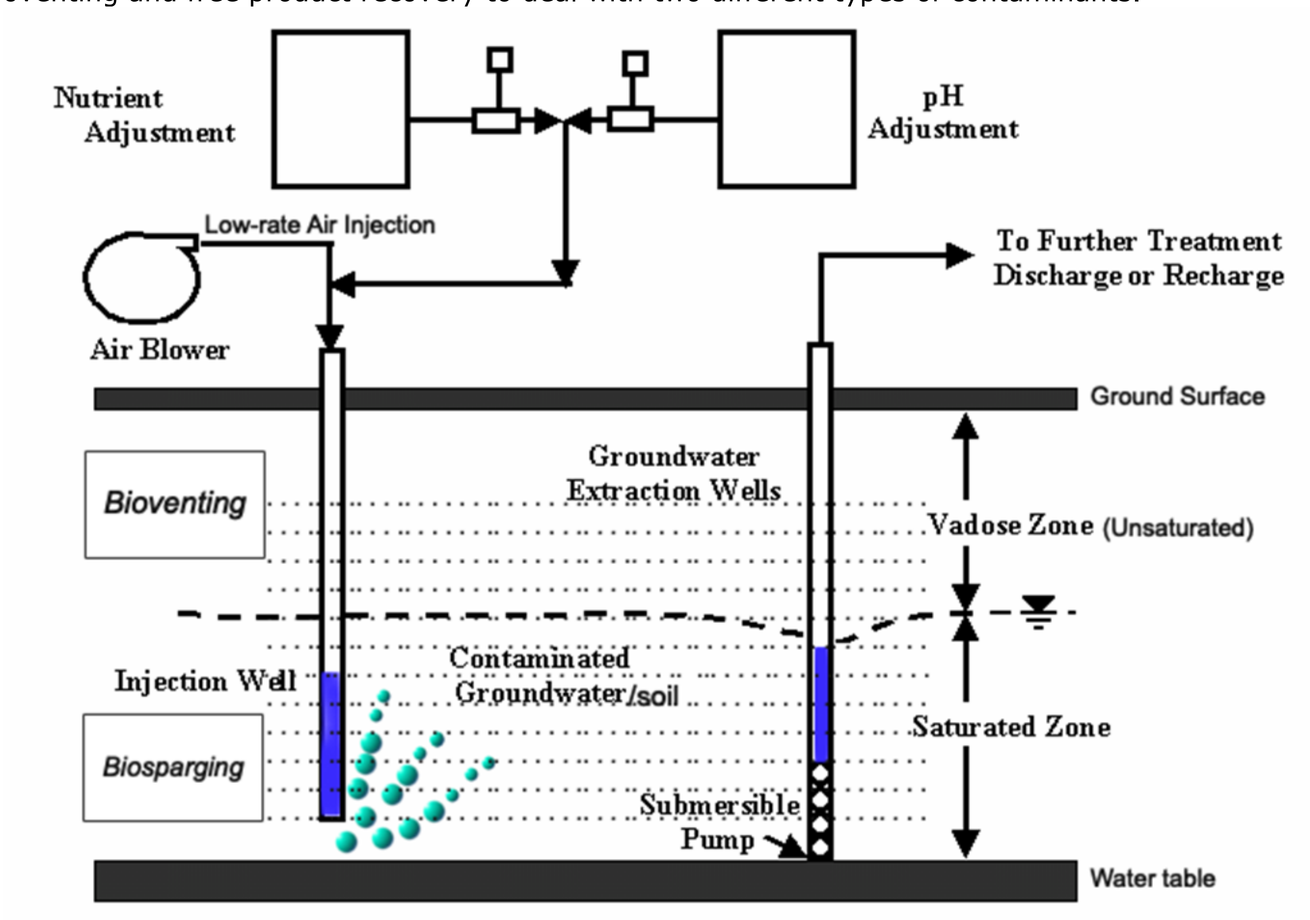

Figure 3. Bioventing and Biosparging System for contaminant remediation *(Adapted from FRTR [23] with modifications)*

2.1.1. Factors affecting the bioremediation application and performance

The outcome and rate of biodegradation of these bioremediation methods differ between *in-situ* and *ex-situ* bioremediation and factors affecting the application of these methods. These factors that influence the application and performance of bioremediation treatment includes**.**

i. *Temperature* - The highest degradation rate is generally visible within the moderate temperatures range of (20–45°C, for mesophilic microbes) or at higher temperatures (>45°C, for thermophilic microbes) depending on the type of microbes. Also, high temperature increases the solubility of pollutant (in the case of crude oil contaminant) and makes contaminants bioavailable for degrading bacteria while low temperature delays the biodegradation process. But extremely high temperatures are disastrous for bioremediation as it's unfavourable for dehydrogenases and could lead to the death of microorganisms.

ii. *Soil pH* is another factor which could be sensitive for the application of bioremediation since the activities of microorganism are a factor of the toxicity of the contaminated environment; moderate pH is favourable for the process. However, the removal of most metallic pollutants depends on pH [24-25].

iii. *Aerobic/anaerobic condition*: the contaminated environment must be aerated, or oxygen supply should be ensured, but some microorganisms (sulfate-reducing bacteria, SRB) operate favourably anaerobically. Thus, great care should be taken to identify the bacteria present and the condition for operation.

iv. *Availability of nutrient or energy source*: a constant supply of nutrients rich in N and P as carbon or energy source to stimulate and enhance the activities of the microorganisms should be ensured for optimum biodegradation rate and performance [26].

v. *Nature of contaminant*: the type of pollutant (Crude oil) involved is a determining factor for the rate of biodegradation. The rate of biodegradation varies according to the type of contaminant. Identifying the type of contaminant provides a platform for the deployment of the bioremediation method best suited for the remediation of such contaminants. The biodegradability of hydrocarbon showed that lower hydrocarbons are easily degradable. Compounds like high molecular wt. polyaromatics (PAHs) may be very difficult to biodegrade [18]. Also, non-biodegradable contaminants (metals) may pose a challenge to the application of bioremediation.

vi. *Bioavailability of contaminant*: availability of contaminants is an essential factor that ensures the feasibility of bioremediation. Contaminants and substrates must be available for contaminant degrading bacteria for microbial metabolism which is a prerequisite for biodegradation [26].

vii. *Nature of the contaminated site*: the site condition is crucial for bioremediation applications. These involve soil type, soil properties (physical and chemical) and indigenous microbial population present and other intrinsic site features. These factors determine the extent of biodegradation of the contaminant.

viii. *Microbial communities*: the indigenous microbial community, coupled with the augmentation of a bacteria strain or consortium is more effective than a single strain or indigenous microbial community alone. According to Yuniati [18], the selection of bacteria strain or consortium must be based on traits with different abilities that will be advantageous to the selected site properties for effective performance.

ix. *Concentration*: the concentration of the contaminant is a determining factor for the rate of biodegradation. The high concentration of the contaminant is detrimental to the microorganisms as it slows microbial metabolism and reduces the rate of biodegradation. Also, at a very low concentration, there is a possibility of non-bioavailability of contaminants to the contaminant degrading bacteria which drastically reduces the biodegradation efficiency. Since most microorganisms are sensitive to high concentration which most times affects metabolism and can lead to the death of the microorganism present, thus, selection of apt microorganism is an important factor to consider which depends on the concentration of the contaminant on the site to be treated [26-27].

x. *Inhibitors*: the presence of possible inhibitors tends to hinder the bioremediation process, which will help to determine whether to apply the bioremediation technology to the contaminated site.

xi. *Seasonality and Plant type*: Bioremediation method especially, phytoremediation progress can be affected by seasonal variations, which is dependent on the location of the contaminated site. Its effectiveness will also be truncated by other climate factors. Also, the availability of contaminant tolerant/resistant plant species is essential for the success of this bioremediation technique [22].

2.1.2. Bioremediation methods for the treatment of crude oil contaminated soils

In recent times, petroleum hydrocarbon has become one of the major pollutants of the ecosystem due to its widespread usage and spill [28-29]. The bioremediation technique has successfully been applied for the treatment of soil contaminated with organic contaminants. This technique is recently receiving unlimited attention sequel to its cost-effective and eco-friendly nature. The objective of any soil treatment method is not only to adequately remove the contaminant but also to revive the organic content of the soil [30-31].

2.1.2.1. Bioattenuation and biostimulation treatment of crude oil contaminated soils

Bioattenuation is the biodegradation approach that is dependent on the natural environmental factors to revive and sustain the growth of the microbial communities to enhance the natural biodegradation of pollutants with human intervention aside from monitoring the degradation rate [30]. Biostimulation entails the adjustment of environmental parameters using organic and inorganic supplements to boost or stimulate the activities of microorganisms in the soil to improve the biodegradation rate and pollutant removal.

Liu *et al.* [21] investigated the biostimulation of contaminated soil using aged refuse (domestic waste) from landfills. The experiment conducted in 1L plastic tubes of three experiments with each triplicated comprised of bioreactors containing contaminated soil and sterilized aged refuse (SAR); aged refuse (AR) treatment and the controlled treatment at varying loading ratios. The result recorded that AR gave the highest biodegradation efficiency of 89.83% (i.e. reduction from 47.28.mg/g initial concentration to 2.46mg/g), an increase in the pH was observed from 6.35 to 7.67 and increased the soil organic matter content from 6.1% to 9.5%. These dilute the total petroleum hydrocarbon (TPH) by half, decreasing the soil ecotoxicity [32]. The SAR and controlled experiment offered 74.64% and 22.40% representing a reduction from an initial concentration of 47.28mg/g to 6.13mg/g and 36.69mg/g respectively. The result showed the potency of organic substrate in the reduction of TPH in the polluted soil. Also, the reduction efficiency of domestic waste was investigated by *Gallego et al.* [33] on diesel contaminated soil using domestic sludge from wastewater plants through the investigation of potential in-situ technique by the study of microbial biodegradation. The result reported 90% biodegradation efficiency after 45 days using inorganic nitrogen (N) & phosphorus (P) while the addition of sludge increased the biodegradation efficiency to an appreciable extent. The microbial study indicated the presence of contaminant degrader *Acinetobacter sp*. degrading most of the contaminant of approximately 40000 L of diesel fuel released.

Ling and Isa [34] also recorded high degradation efficiency in the evaluation of the efficacy of composting and sewage sludge for the remediation of refinery oil sludge and also the optimum ratio of polluted soil to sewage sludge amendment to facilitate the degradation of refinery sludge was determined. The result showed that optimum oil and grease efficiency was achieved after a 9-week study period at 65% degradation efficiency under low temperature for an optimum ratio of 1:0.5 (v/v; soil to sewage sludge ratio) but the treatment failed to remove most of the recalcitrant (heterocyclic) components. A similar removal percentage was reported by [35] where the use of sewage sludge accounts for the degradation efficiency of 45% to 60% of oil in contaminated soil for a 5 to 10 weeks study period. It reduced hydrocarbon classes $C_{17} – C_{21}$, $C_{22} – C_{25}$, $C_{26} – C_{29}$, $C_{30} – C_{36}$ at 37%, 22%, 69%, and 62% reduction efficiency accordingly within 10 weeks study period. It can be inferred that the organic nutrient amendment (supplementation) can improve, enhanced, and reinforce microbial activities to foster biodegradation efficiency [36-37].

However, a study by Obiakalaije *et al.* [37] to authenticate the efficacy of organic animal waste supplement (goat manure, poultry dropping, and cow dung waste) for the remediation of crude oil polluted soil reported that organic substrate was potent for the decrease in TPH concentration in the polluted soil with biodegradation rate of 70.7% to 87.1% from the three amendments with the highest efficiency shown by goat manure (87.1%), followed by poultry dropping (78.6%) and then cow dung (70.7%) as against the natural attenuation which gave 32.1% after 28 days period. The study recorded an increase in total microbial (heterotrophic bacteria) count in all samples treated with animal waste with the highest population observed in the sample amended with goat manure. The existence of indigenous microorganisms in animal waste accounts for the significant increase in the total heterotrophic and hydrocarbon utilizing counts [37].

Similarly, organic and inorganic compounds were used in the study by Ofoegbu *et al.* [38] to determine the rate of biodegradation using these supplements for 40 days. The experiment was performed at varying loading ratios of organic and inorganic fertilizers singly and in combination with a ratio of 100 or 50:50 respectively added to the crude oil polluted soil at 2%, 4%, and 6%(w/w). The result showed that the combination of cow dung (organic) and inorganic fertilizer gave the highest degree of biodegradation efficiency of 84.62% for 2% crude oil contaminated soil followed by NPK, cow dung, (CD and PKHA) with PKHA having the least removal efficiency. The study also reveals that the rate of TPH removal is a factor of the volume of contaminants or the degree of contamination, thus, the higher the contaminant the slower the rate of biodegradation and vice versa and also the significant difference in biodegradation rate of the organic and inorganic stimulants was elucidated [38].

Also, the study by Chijioke-Osuji *et al.* [39] showed similar results of 88% biodegradation efficiency with the combination of poultry manure (organic) and inorganic fertilizer (NPK 15:15:15) after 112 incubation period which elucidates the discrepancy between soil amended with supplement and natural attenuation. The dependence of biodegradation rate on the degree of contamination was reaffirmed by Naowasarn and Leungprasert [40] in the remediation of oil-contaminated soil using chicken manure (organic supplement) as the result of 5% contaminated soil treatment gave the highest TPH reduction efficiency of >60% more than the 10% and 20% treatment at equal addition of organic supplement after 42days study period. It can be inferred that a high concentration of contaminant contributes to the decrease in the biodegradation efficiency of hydrocarbons [41]. In contrast, the study by Ani *et al.* [42] and Obiakalaije *et al.* [37] reported that low concentration of contaminant showed a significant decline in the rate of biodegradation due to the limitation of the bioavailable target contaminant for the microbial intake which indicated that the available contaminants are practically insufficient for the biodegradation activities. Invariably, the optimum concentration of the contaminant is required as the TPH removal efficiency depends on the availability of nutrients for microorganism utilization [37, 43].

Imafidon and Ogirigbo [44], Amhakhian and Faleke [45] and Omokaro [46] also showed the efficacy of cow dung (organic) to stimulate soil microbial activities which are facilitated by the amendment of extra nutrients (organic or inorganic) to increase the biodegradation efficiency of organic pollutant. This can be attributed to the ability of the organic substrate to establish a stable environment for microorganisms to thrive and enhance TPH removal in the polluted soil [41, 45]. Imafidon and Ogirigbo [44] illustrated the aforementioned with the addition of nutrient agar to cow dung and NPK which increased the TPH removal efficiency by 10% (from 61.93 to 72.13%) and that of NPK by 3% (from 52.85 to 55.50%) in 10 weeks. This practically falls within the biodegradation efficiency recorded by Isitekhale *et al.* [47]. Invariably, Ramsay *et al.* [48] noted that the amendment of organic fertilizer will have an appreciable impact on the growth of hydro carbonic degrader microbes in the soil which enhances bioremediation efficiency.

According to the study by Margesin and Schinner [49] inorganic fertilizer decreased the initial concentration of crude oil contamination from 4000mgkg^{-1} soil matter to 380 - 400mgkg^{-1} after 155 days of incubation in the treatment of oil-polluted soil. Sequel to the result of the study, approximately, 30% of the TPH was eliminated by an abiotic process while 60 – 65% was attributed to microbial degradation. The addition of supplements can significantly improve the biotic and abiotic conditions of the soil polluted with petroleum hydrocarbon [44].

Ani *et al.* [42] analysed the optimization process of the organic substrate, goat dung as a potential co-additive in the remediation of crude oil-polluted soil. The study reported that the introduction of goat dung increased the biodegradation efficiency (70–75% of 130g/L initial concentration) and the pH. The addition of organic substrate tends to enhance dehydrogenase activities which promote biodegradation efficiency and increases the pH [45, 50]. As the neutral pH of 7 recorded the highest removal efficiency, Ani *et al.* [42] noted that an optimal pH of 7 provides a favourable condition for the growth of microorganisms which will, in turn, enhance the remediation of the process. It has been reported that extreme pH values are harmful to microbial growth and activities and reduce their ability to comfortably degrade target contaminants [51]. It showed that organic substrate, goat dung is an effective co-substrate and viable approach for the treatment of oil-polluted soil having contributed to little or no risk, to 70-75% reduction efficiency of TPH concentration in the contaminated sample after 56 days period [42].

Adekunle *et al.* [50] investigated the degradation of hydrocarbon contaminated soil using sheep waste and goat waste compost as an organic stimulant. The result of the study recorded that biodegradation can be influenced by soil type, period of application, and quantity of compost or supplement [18]. The influence of these factors was experienced in the remediation of TPH polluted soil using the same organic substrate, cow dung as investigated by Amhakhian and Faleke [45] and Imafidon and Ogirigbo [44] recorded biodegradation efficiency of 89-98% after 28 days and 61.93% after 70 days period respectively. The discrepancies in the removal efficiencies recorded by different researchers justified the fact that the TPH removal efficiency is dependent on certain conditions [50]. There was also a decrease in the acidity of the polluted soil supplemented with sheep and goat composting indicated that organic substrate can increase the pH of soil polluted by petroleum hydrocarbon (PHC) at low cost and without visible risk of recontamination of the sample by the substrate [42, 50, 52].

In the study to authenticate the potency of slurry phase biological method and land farming for the remediation of crude oil polluted soil by Kuyukina *et al.* [41] reported that slurry phase bio-treatment was able to achieve a biodegradation efficiency of 88% after 2 months of the study period and application of land farming as showed 300 – 600ppm/day reduction in oil concentration. Similarly, Einawawy and Salba [53] reported that land farming was successful in the treatment of TPH contaminant with 80% contaminant removal. The manpower requirement of this approach is enormous as the 120m farming plot under investigation took 15 months to achieve an appreciable biodegradation efficiency [53]. Land farming technology has been widely used for its cost-effectiveness, but the physical, chemical and biological components of this method may pose serious damage to the entire remediation process if not properly managed. The dominant contaminant removal technique involved in land framing is the volatilization of low weight volatile compound in the course of the early stage of the treatment process, leaching and remaining recalcitrant hydrocarbon can pose serious health and environmental challenge to the rehabilitation workers when designing the land farming technology on the contaminated site. Also, the large expanse of land, flexibility, manpower, and effectiveness of the approach at high TPH concentration (> 50,000ppm) are among the drawback of this approach [54].

Adekunle [55] studied the remediation of soil polluted with Nigeria petroleum products using composted municipal waste and the growth of maize as a risk assessment on soil quality and remediation evidence. The result of the research recorded that the treatment of petroleum-contaminated soil increased pH and electrical conductivity and decreased the TPH at 40-75.8% efficiency with toxicity reduction from 100% to 16.2% after 21 days of treatment. Maize growth was observed in the composted remediated soil which signified the evidence of soil restoration for agricultural purposes. Seed germination in the soil that received no treatment suggests that a certain degree of crude oil contamination supports plant growth. Kuhn *et al.* [56] supported this with the treatment of Kuwait crude oil contaminated soil where the germination and growth of tomato (*Lycoperisucum esculantum*) was feasible at 0% - 0.36% crude oil contamination with toxicity and growth inhibition recorded at ≥0.48%. However, the Nigerian crude oil used by Adekunle [55] supports maize seed germination up to 5% contamination. It can be inferred that oil phytotoxicity can vary with location, type of crude oil, species or type of plant and/or climatic conditions [55].

2.1.2.2. Bioaugmentation of crude oil contaminated soils

Bioaugmentation is the process of introducing isolated bacterial strain or microbial consortium or genetically engineered bacteria with defined catabolic attributes to accelerate the dehydrogenase activities, increase biodegradation efficiency to produce the expected outcome [57-58]. Bioaugmentation is favourable in polluted soil that has possibly undergone bioremediation but still pose an environmental risk since indigenous microorganisms failed to accomplish the biodegradation of contaminant during the process. Hence, this bioremediation is posed to improve the rate of pollutant removal through the injection of biodegradable bacteria consortium or strain of microorganisms. The type of microorganism to be used in the decontamination process is dependent on the type of contaminant, physiological, and microbial metabolic activities to comfortably degrade contaminants. Since there's no single microorganism that can degrade all the contaminants present in a contaminated sample, researchers have studied the ability of most microbes to remove contaminants and most studies have focused on bacteria and fungi.

Olukunle and Oyegoke [59] investigated the bioaugmentation of crude oil using fungi isolated from cow dung polluted soil. The study identified 16 fungi from the contaminated soil which possesses contaminant degradation ability with *Trichoderma viridae (66.2% TPH reduction efficiency), Aspergillus flavus and Varicosporium elodeae (40% TPH reduction rate)* species demonstrating the best degradation ability in 15 days study period. The study by Kristanti *et al.* [60] on the bioaugmentation of crude oil using fungi (white-rot fungi *Polyporus sp.* S133) and the effect of three nutrients (glucose, polypeptide, and wood meal) were evaluated. The outcome of the treatment reported that appreciable TPH removal efficiency of 93% was feasible with the addition of 10% kapok. In the same vein white-rot fungus (basidiomycetes fungal isolate *Armillaria* sp. F022) was applied by Hadibarata and Kristanti [61] in biodegradation and metabolite conversion of pyrene. The result of the treatment reported pyrene concentration, <19% after 30 days of incubation as the high removal efficiency correlated with the degree of depletion of carbon source (glucose) used for the treatment. These demonstrated the ability of white-rot fungi to remediate crude oil contaminated if properly developed [60].

Similarly, on mycoremediation, Winquist *et al.* [62] investigated the removal efficiency of recalcitrant PAHs using fungi inoculum; strain *Phanerochaete velutina* which showed a significant reduction of in TPH concentration most especially PAHs (at 96% of 4 - ring PAHs and 39% of 5 -& 6 – ring PAHs) removed from the polluted sample as against the inoculated treatment stimulated with green waste (biostimulation only) which recorded 55% reduction of 4 – ring and only 7% of 5 & 6 – ring PAHs in 3 months treatment period for the laboratory scale experiment. The field-scale study observed an almost similar biodegradation efficiency for the *P. velutina* inoculated and inoculated treatment recorded 96% of 16 PAHs degraded in 3 months. This indicated that bioaugmentation using fungi has the capacity of biodegrading TPH from contaminated soil and restoring the organic content of the soil, although most of the study on fungi remediation ability is still at the laboratory stage [60, 62-63].

The study by Benyahia and Embaby [64] to investigate the relative potency of bioaugmentation (BA) and biostimulation (BS) noted that crude oil contaminated soil requires a combined application of BS and BA for effective remediation of contaminated soil. The result of the study showed that bio-piling amended with BS and BA offered 77% TPH removal efficiency after 156 days study period. The single treatment with bioaugmentation gave 55% removal efficiency and biostimulation with indigenous microbes recorded 23% as against bioattenuation with a 4% biodegradation efficiency. The study reported that the availability of biodegradable contaminants and nutrients can affect the rate of biodegradation [64].

Ghaly *et al.* [65] evaluated the efficacy of nutrient amendment and augmentation of mycobacteria species for the treatment of PAHs and pyrene polluted soil. The findings from the study recorded an increase in the number of cells: 40, 70, 59, and 132 for control, biostimulation, bioaugmentation, and combined biostimulation & bioaugmentation (BST + BAU) respectively during the treatment period. Consequently, the increase in number of cells corresponds to the appreciable degradation of PAHs. Wilson and Jones [66] validated the efficacy of BST + BAU treatment in a study conducted within the temperature range of 20 – 40°C and moisture content of 40 - 60% for PAHs degradation. The investigation recorded the highest PAHs and pyrene removal efficiency of 84% with the combined process of biostimulation and bioaugmentation, followed by bioaugmentation (57.86%), biostimulation (50%) while the least efficiency was recorded with natural attenuation with 37% pyrene removal efficiency after the 14 days study period. The outcome of the study by Abdulsalam *et al.* [67] was in contrast with Ghaly *et al.* [65] experiment where biostimulation using inorganic fertilizer and dihydrogen orthophosphate recorded 75% higher than bioaugmentation (with bacteria consortium) which gave 66% while bioattenuation offered 50% removal efficiency after 10 weeks study period for the treatment of soil polluted with spent motor oil in an aerobic fixed bioreactor. The appreciable removal efficiency rrecoreded by biostimulation can be attributed to the appropriate biostimulant ammendment which contains significant organic components for microbial activities. However, the study by Mao *et al.* [68] to investigate the performance of bacteria consortium for the treatment of PAH contaminated soil reported that the addition of 20% bacteria consortium gave (35.8%) high PAH removal efficiency than the 10% treatment (20.2%) after 90 days of incubation. An increase in bacteria consortium was reported at the early stage of the remediation process and decreases gradually as the treatment progress and the low removal efficiency was due to high concentration of PAH in the sample [68].

Sugiura *et al.* [69] studied the correlation between biodegradability and physicochemical properties of petroleum, which expatiates chemical species of petroleum that are recalcitrant to biodegradation using bacteria consortium. In the study, a microbial bacteria consortium SM8 isolated from sediment and sub-cultured in crude oil medium and *Acinetobacter* isolated from the Pacific Ocean was used for the experiment for the treatment of four crude oil samples from different locations. The result showed that both strains of bacteria degraded oil samples at different rates with *Acinetobacter sp. T4* recording 19 – 34% and SM8 consortium 12 – 20% biodegradation efficiencies after 28 days of treatment. Although *Acinetobacter sp.* T4 recorded an appreciable biodegradation efficiency than SM8 sp., it was reported that SM8 consortium recorded significant degradation of naphthalene, phenanthrene, fluorene, dibenzothiophene and their structural analogue than *Acinetobacter sp.* T4 was more effective on alkyl chains, but polycyclic aromatics were recalcitrant to this bacteria strain (SM8 sp.).

2.1.2.3. Combination of biostimulation and bioaugmentation

In a study by Qiao *et al.* [70] to evaluate the effectiveness of different amendments using NPK, fertilizers, humic substances, organic industrial waste (NOVOGRO) and yeast-bacteria consortium for enhancing the treatment of PAH from polluted soil (up to 6% hydrocarbon)0 recorded that biostimulation, (the mixture of NPK, HS, and NOVOGro) showed the greatest efficiency of TPH removal in 90 days study period. Also, the addition of exogenous oil-degrading bacteria had a minimal effect on the biodegradation efficiency of contaminants but the introduction of external yeast bacteria consortium (bioaugmentation) showed a significant increase in removal efficiency of more recalcitrant PAH while bioattenuation offers the least result. This proved that combination biostimulation (organic and inorganic supplement) and bioaugmentation (use of microorganism consortium) are more efficient in the treatment of crude oil polluted soil; the removal of recalcitrant (PAH) hydrocarbons and decrease in toxicity of contaminated soil, since the augmentation with yeast bacteria facilitated the removal of more recalcitrant hydrocarbon. The removal of PAH by yeast bacteria consortium is due to the synergistic impact of bacteria and fungi that exists in the sample which indicated that that the introduction of external microorganism is more efficient than the indigenous organisms in the removal of PAHs citing salient features of microbial consortium as reasons for its potency in the treatment [70].

Assessment of biodegradation ability of bacteria isolated from oil-contaminated soil with the animal waste amendment was investigated by Urhibo and Ejechi [71]. The result of the study showed that the TPH removal by bacteria in the animal waste amendment was more than that of strain from soil contaminated with petroleum and the greatest TPH biodegradation efficiency was recorded with poultry waste, strain *P. Vulgaris* (96.6 – 97.3% as against 80.4 – 95.9%) after 6 weeks of treatment. The high degradation efficiency of the sample with bacteria isolated from animal waste amended with animal waste can be attributed to the ability of the animal waste supplement to act as an energy source for the bacteria which enhanced the biodegradation efficiency unlike the strain without carbon source [73]. The use of bacteria for the remediation of contaminated soil is affected by carbon source and environmental factors which limits its potency and metabolic activities resulting in low biodegradation efficiency. Accordingly, while some bacteria are sensitive to PHC, exposure to PHC may adversely affect their potency and activities, others can utilize the cytotoxic intermediate metabolite and flourish [72-73]. Thus, cleaning crude oil contaminated soil with bacteria strain will be tedious without an energy source to improve the process performance.

The study by Fan *et al.* [74] reaffirmed the potency of the combined systems of biostimulation and bioaugmentation with yeast which recorded a TPH reduction of 83%. Concentration effect contributes to contaminant bioremediation as (low concentration) 0.5% (v/v) gave 96% more than (high concentration 5% (v/v) with 42% biodegradation efficiency. Similarly, records indicated that biostimulation with a stimulant (sludge) and hydrocarbon sulfate degrading bacteria accelerated the rate of biodegradation of TPH and recalcitrant PAH [75]. Also, an investigation by Suja *et al.* [76] on the effect of native microbial bioaugmentation and biostimulation in the bioremediation of TPH polluted soil based on pilot and field study showed that the combined approach is efficient in reinforcing the growth microbial community and dehydrogenase for the treatment of TPH contaminated site as 97% biodegradation efficiency was recorded after 70 days.

In the assessment of bioaugmentation and biostimulation efficiencies for petroleum contaminants, [77] where bacteria consortium (*Acinetobacter, Alcaligenes, Bacillus, and Pseudomonas*) and nutrient supplement. The result of the process indicated that bioaugmentation has the highest efficiencies of degradation with 73.89, 73.76 and 58.31% respectively for 3g, 30g and 60g oil concentration/kg respectively whereas biostimulation boosted 52.11, 58.36 and 43.02% and natural attenuation with 15.33, 15.48 and 13.01% for 3g, 30g and 60g oil concentration/kg respectively after 90 days incubation period. The result inferred that oil concentration of more than 30g/kg is not appropriate for bioremediation to avoid the increase in toxicity which contributes to the inhibition of the process. The concentration of oil among other environmental factors is a crucial function of bioremediation of oil contamination since the efficacy of bioaugmentation is a factor of effective attachment, retention and metabolic population in the bioreactor system which should be equivalent to the oil concentration for optimum performance [77].

2.1.2.4. Bioventing treatment of crude oil contaminated soils

This is a bioremediation approach that cost-effectively removes light and middle hydrocarbon distillates from the unsaturated zone through the combination of soil venting and improved bioremediation. The removal ability of bioventing is by direct air injection through the vadose zone to revive, revitalize, resuscitate and promote aerobic respiration within the contaminated soil environment which enhances biodegradation of more volatile hydrocarbons [78]. Bioventing system is structured to enhance sufficient oxygen supply to ventilate the vadose zone and activate oxic condition in the contaminated site, usually operated at low flow rates with designs and configuration different from soil vapour extraction (SVE) [79].

Mao *et al.* [80] evaluated the treatment of crude oil polluted soil by bioventing and composting technology application where inorganic fertilizer was used as a stimulant for the bioventing in three varying ratios; 8:2, 7:3, 5:5 for contaminated soil to organic fertilizer (dry weight). The result showed that 45% of TPH was removed from the soil in 40 days period from an initial concentration of $7.0x10^4$mg/kg. The highest reduction efficiency of 45% was observed with the 7:3 treatment, which attributes to the high concentration of the contaminant. Volatilization removal was less than 0.1 which suggested that degradation was most active due to the bioremediation process. Similarly, Lee and Swindoll [81], conducted a laboratory experiment on the feasibility of bioventing applications for the treatment of hydrocarbon (light and heavy). The study carried out using three treatments was operated for bioventing, organic nutrient, and moisture; bioventing without organic nutrient and moisture, and the controlled experiment. The result recorded that after 90 days of treatment at an operating temperature of 22^oC, bioventing was the most effective TPH removal from the contaminated soil. Bioventing with organic matter and moisture boosted 98%; bioventing without organic matter and moisture gave 83% and the controlled experiment showed the least biodegradation efficiency of 29%. Bioventing with nutrient was effective in the removal of BTEX and recalcitrant PAHs (96%); heavy hydrocarbon (75%). The result showed that bioventing is apt for the treatment of hydrocarbon ranging from light to medium (gasoline and diesel) to heavy hydrocarbons (such as fuel oils and other volatile, non-volatile HC and PAHs); which inferred that nutrient amendment increased the removal efficiency of bioventing [79, 81].

This was further justified in a study by Møller *et al.* [82] to evaluate the bioventing of diesel oil-polluted soil using supplements and comparison of removal efficiencies based on actual oil concentration and respirometric data. Bioventing was supplemented with nutrients (Nitrogen and Phosphorus added as a mixture of $NaNO_3$, KNO_3 and $NaHPO_4$ dissolved in water to give a C: N: P ratio of 120:10:1 based on concentration content of oil) and inoculated with oil-degrading bacteria (isolated from an enrichment culture of bacteria from diesel contaminated soil). Similarly, bioventing treatment of phenanthrene-polluted soil using optimum conditions of mineralization: humidity: 60% and different C/N/P ratio of 100:20:1 respectively was effective as it enhanced the removal efficiency after 7 months of study. The result indicated that nutrients and inoculation increased the rate of bioremediation of contaminated soil [79, 81, 82] and respirometry test has no appreciable impact on the removal of diesel oil, thus, removal of contaminant was done by bioremediation [82]. The ability to achieve an enhanced bioventing biodegradation with nutrient addition was in contrast with the investigation by Dupont *et al.* [83] where the amendment nutrient (moisture) was insignificant for the increased rate of biodegradation of fuel contaminated soil. However, Bulman et al. [84] demonstrated that nutrients amendment to bioventing rendered an appreciable increase in the rate of degradation of TPH which suggested a further study on the nature and type of individual nutrient required for each contaminant for the bioventing process to foster biodegradation rate since some additives (nutrients) can reduce or hinder biodegradation or constitute to the increase in toxicity of the sample after the treatment [79].

Eslami and Joodat [85], studied the bioremediation of oil and heavy metals polluted soil using bioventing – bio-sparging and phytoextraction (plant assisted bioremediation) techniques. The result of the study showed that a combined process of venting and bio-sparging rendered the highest efficiency of biodegradation, reducing 60% of the contaminant in 40 days period while the phytoextraction technique was effective in reducing heavy metal contaminants up to 50% after 50 days of study. The process indicated that air-injection nourishment to the system improved the degradation rate by providing an optimum soil medium for the remediation process. In bioventing and bio-sparging, the removal efficiency of ethylbenzene was more than that of pyrene, attributed to the discrepancies in their molecular structures [85]. Ethylbenzene, which is of the BTEX family possesses one cyclic hydrocarbon, different from pyrene (of PAHs group) with multiple cyclic hydrocarbons elements – which made biodegradation of pyrene slow and more difficult than BTEX. Also, the less complexity of the BTEX family attributes to the self-bioremediation of contaminants containing ethylbenzene, unlike pyrene which falls under the recalcitrant PAHs. [85-86].

Lee *et al.* [87] monitored the bioremediation of diesel fuel in the bioventing process using an in-situ respiration rate. The experiment comprised of 5kg of soil contaminated with 8000mg/kg of petroleum hydrocarbon was conducted in a column, with variation in flow rate; where one received continuous venting and the other column received venting for 6 hours/6 hours resting during the 5 months study period. The result indicated that there is no apparent variation in the biodegradation efficiencies between the two columns with varying flow rates when measured with an online measuring system of respiration rate. The result supports the study by Thomé *et al.* [88] where there's no significant difference in the biodegradation rate at varying air flow rates and airflow intervals at 2, 4 and 6L/m (corresponding to 0.36, 0.82 and 1.4kPa) at 1-hour flow every 24, 36 and 48 hours for 15, 30 and 60 days respectively. From the result, the highest biodegradation rate was recorded at 85% for bioventing and 64% for natural attenuation. It suggested that bioventing will be more economical if the lowest flow rate (2L/m) and highest flow interval will be considered for the bioventing of contaminated soils while increasing bioventing flow interval and rate is not justifiable due to high operation cost [88]. Volatilization was not considered in the process because the contaminant (diesel) has fewer volatile components and considering low operating temperature, and low flow intensities, these components have negligible effects [80,89].

However, the study by Frutos *et al.* [79] reported the ability of the bioventing technique to significantly remove 93% of phenanthrene (PAHs) from 1026mg/kg initial concentration to 74mg/kg in 7 months period. The Ecotoxicity test indicated that the residual toxicity (which pose an ecological challenge) obtained at the end of treatment was basically due to C/N used for the optimization of the system and not low phenanthrene concentration, which suggested that type of nutrient amendment should be considered in the bioremediation process [84]. Also, the treatment showed a decrease in degradation efficiencies with a corresponding increase in the treatment period where the highest rate of biodegradation was recorded between months 1 – 3 while gradual decline commenced from month 4 with a significant decrease in biodegradation efficiency from month 5 till the 7th month of the study period. It can be inferred according to Frutos *et al.* [79] that the removal efficiency is a function of time as it tends to increase or decrease significantly with time.

The application of bioventing and bio-trickling filter technologies for soil remediation was investigated by Magalhães *et al.* [90]. In the study, the soil was artificially polluted with aromatic hydrocarbon (toluene) of 100mg/dm^3 and 500mg/dm^3 homogeneously to attain the desired soil contamination and the mineral medium was added to the soil for moisture regulation at 10%. Microbial inoculum culture of 10cm^3 was added to the two bioreactors for bioventing and combined bioventing and bio-trickling after 6 days. The result should that bioventing and combined bioventing and bio-trickling gave the same rate of biodegradation of 99% removal of toluene (at an initial concentration ranging from 2 to 14mg/g soil) after 20 days study period with toluene attributed removal to the combined effort of biodegradation and volatilization as against 80% removal efficiency recorded for the untreated sample. Sequel to the volatile nature of the toluene, volatilization was able to eliminate some volatile components of the contaminant unlike the case of non-volatile diesel contaminants as earlier reported [90]. Also, the combination of the two processes rendered a significant removal efficiency of 99% toluene removal from the soil. The process which was not carried out with high organic load to determine the extent of biodegradation rate when bioventing is compared to biotrickling posed a limitation to the study. However, Chou and Wu [91] reported that the treatment of toluene using the combination of BVT and BF showed a higher rate of biodegradation of 90% in 121 days at a high organic load of 30g/h which inferred that the combination of these techniques can be effective for the remediation of toluene from a polluted site.

Agarry and Latinwo [92] investigated the application of bioventing and wastewater for the remediation of diesel polluted soil in a microcosm system containing 1kg soil spiked with 10% (w/w) crude oil to achieve desired contamination and monitored for 28 days. The result observed that a combination of brewery waste effluent supplement and bioventing technique gave the highest TPH degradation rate of 91.5%; bioaugmentation and biostimulation with brewery waste effluent recorded 78.7% removal efficiency and 61.7% for bioventing. The natural attenuation gave the lowest rate (≤40%) of diesel removal since the treatment received no amendment or supplementation. Also, the increase in total hydrocarbon-degrading bacteria (THDB) count throughout the treatment period in all the systems was observed with the highest bacteria growth visible with the combined bioventing and brewery waste effluent approach. It was also reported by Thome *et al.* [88] combined biostimulation and bioventing was more effective than biostimulation or bioventing used alone. Brewery waste tends to increase the nutrient level and microbial density in the soil thus acting as bioaugmentation and biostimulation agent. A similar trend was recorded by Muskus *et al.* [93] where organic components (animal waste) acted as bioaugmentation and biostimulation agent to facilitate the remediation process. The use of bioventing and biostimulation/bioaugmentation was suggested to be an environmentally sustainable approach for the remediation of the natural ecosystem [88,92].

3. *Pseudomonas aeruginosa* as an effective bioremediation tool

The genus *Pseudomonas* is summarily described in terms of phenotypic and genomic features of its member species and can utilize varieties substrates (organic and inorganic), survive different environmental conditions and may grow in simple media, and their nutritional flexibility enables them to survive in contaminated environs which may be toxic to other bacteria as found in *Pseudomonas* studies. These characteristics suggest *Pseudomonas a*s a viable agent for bioremediation purpose [94].

To improve the rate of biodegradation of hazardous organic compounds in the terrestrial and aquatic environment, microorganism application has proven to be effective in reducing toxic material concentration so far. However, the study revealed that microorganisms that exhibit or show chemotaxis towards the environment tend to perform better than non-chemotaxis organisms that attribute different degrading microbes to variation in the biodegradation efficiencies. It has been recorded that oil-degrading microorganisms like E, Colin Salmonella, *Pseudomonas aeruginosa, P.Putida, Bacillus Cereus, Myxococcus sp., Rhizobium and Azopirillium sp.* have some appreciable chemotaxis behaviour which contributes to their performance in the bioremediation treatment [95].

P. aeruginosa is a gram-negative bacterium, gammaproteobacterial, aerobic, rod, and family Pseudomonadaceae that can withstand heavy metals such as copper, cadmium, chromium, nickel [96]. The deposition of metals in soil in high concentration is undesirable for plant growth and development due to its non-biodegradable nature. This has made the bioremediation of heavy metals a difficult process. Some bacteria have special morphology and can absorb/accumulate metals on their cells [97]. Because of its abundance, availability on earth, cost-effective and eco-friendly nature, the microbe is suitable for remediating metal-polluted soils. Some of these bacteria have been used in the bioremediation process to treat heavy metals and many of these bacteria that have proved to be active in the treatment for bioremediation include the organisms *Pseudomonas, Bacillus, Escherichia, Micrococcus, and Streptomyces*. They develop in the presence of heavy metals by rendering metal binding [98] with functional groups and metal chelating agents present on the cell wall.

Chemotaxis is a systemic, complex mechanical process whereby bacterial cells detect significant (low or high) concentration changes and respond behaviourally to the change, then adjust to tolerate or adapt to the new change in chemical stimulus concentration. Reaction to this situation differs depending on microorganisms where the chemotaxis may be reacted positively when the microorganism moves in the direction or absorbs the compound or moves away or repelled by the compound when it responds negatively to the circumstance. With the above, the response to chemotaxis includes attractant or repellent concentration gradients. Some organisms like *Pseudomonas sp.* are chemotaxis, which accounts for their effectiveness in the bioremediation process [99].

3.1. Bioaugmentation of hydrocarbons using *P. aeruginosa*

Glycolipids are biosurfactants of low molecular weight, to which hydrocarbons bind to long-chain aliphatic acids or lipopeptides. Glycolipids such as rhamnolipids, sophorolipids, trehalose lipids are disaccharides that have long-chain fatty acid acylated. Among the glycolipids known are the most versatile and studied rhamnolipids developed by the species *Pseudomonas* – consisting of two moles of rhamnose and two moles of ß-hydroxy-decanoic acid [100]. It was noted that rhamnolipids would individually reduce the water surface tension from 72mNm^{-1} to 25 – 30mNm^{-1} at a concentration of 10 – 200mg/L^{-1} [100].

3.1.1. Biosurfactant production

Biosurfactants are synthesized during the growing time when they enter the stationary growth stage. Biosurfactant emulsifier production has caused cell proliferation and represents the stationary stage of growth [101]. Since these species may use crude oil as an energy source and at the same time degrade selected fractions of hydrocarbons and become hungry once hydrocarbon range has been depleted [102].

With respect to orientation, the cell surface of the microbial cell may be more hydrophobic if the biosurfactant is cell-bound. This is evident in Pseudomonas aeruginosa, where the hydrophobic aspect of the cell surface is greatly enhanced by the existence of cell-bound rhamnolipids – in contrast to Acinetobacter, where the cell surface is decreased by the existence of cell-bound emulsifiers [103]. It can be inferred that microbes can use the biosurfactant they generate to curb their cell surfaces, characteristics, connect or remove themselves from the surface according to their requirements.

Rosenberg *et al.* [103] proved this in their analysis where bacteria degrading oil *A. calcoacticus RAG-1*, which uses n-alkenes as a carbon source for growth and metabolic activity, suffered malnutrition as hydrocarbon options were reduced while oil droplets are still abundant in aromatics and cyclic paraffin. The famine prompted the production of the emulsan mini capsule. Such emulsifiers, emulsion releases starving cells from the depleted oil droplet of n-alkanes by forming polymeric films around the depleted droplet. The depleted oil droplet is classified as an empty (exempt from n-alkanes) energy source as the cell is desorbed. The emancipation of the cells from the depleted oil droplets motivates them to search for new oil droplets or nutrients. Oil droplet now has a hydrophilic outer surface after depletion which makes it difficult for the bacterium to bind or bond to any used droplets. Bacteria detachment from depleted oil droplets by emulsifiers increases dehydrogenase and enhances bacteria's free movement in a bid to mobilize the necessary fraction of hydrocarbon for an energy source. Therefore, this cycle improves the productivity of biodegradation and facilitates the bioremediation of polluted areas with hydrocarbons [100].

3.1.1.1. The use of biosurfactant in bioremediation of contaminated soils

There are two methods or mechanisms adopted by biosurfactant developed by *Pseudomonas aeruginosa* in the treatment of soils polluted by hydrocarbons [100], which includes increasing hydrophobic water surface area-insoluble substrate and enhancing the bioavailability of hydrophobic compounds

i. Increased hydrophobic water surface area-insoluble substrates.

The rate of growth of oil reducing hydrocarbon bacteria may be influenced by the interface surface area that exists between water and oil. The limitation of the surface area can result in arithmetic, rather than exponential biomass production. Emulsification is a cell density-dependent occurrence, i.e. increased cell numbers increase extracellular products. The concentration of cells in an oil open system like the hydrocarbon polluted environment of water was never sufficient to solubilize the oil. Any solubilized oil is dispersed in water which attributes to its bioavailability to the emulsifying producing strains and the indigenous competing microorganisms. Emulsifiers do not actively participate in the biodegradation process, rather they produce an enabling environment for the degradation of hydrocarbon by producing macroscopic emulsion in the bulk liquid [100].

ii. increased bioavailability of hydrophobic compounds

The bioavailability of hydrocarbon fractions, particularly PAH, depends on solubility, as low solubility tends to reduce the availability of these fractions of hydrocarbons to degrading bacteria. These pose a grave challenge to the successful degradation of hydrocarbons. The poor solubility (which increases surface sorption) of high molecular hydrocarbons is due to their recalcitrant existence-resulting in substrates being restricted to degrading bacteria. This is because bonding organic molecules to surfaces prevent biodegradation. Biosurfactant increase growth rate on the attached substrate by desorbing them from the surface or by enhancing their apparent water solubility [100, 104-105].

Biosurfactant's stability, eco-friendly, and selectivity nature accounts for its effectiveness as the chemical and synthetic surfactant, by increasing the bioavailability of the hydrophobic compound for the hydrocarbon bioremediation process. In mobilizing insoluble molecules and ensuring their availability for bioremediation, surfactants that can conveniently reduce the interfacial are effective. Biosurfactant (emulsifiers) may serve as a substratum (additive) to facilitate the process of biodegradation when produced by microbes. It is possible to introduce bacteria that are capable of overproducing bioemulsifiers which can diffuse in the soil or transfer to bacteria in close contact while participating in biodegradation [100].

Bioaugmentation (use of microorganisms) can be affected by temperature. Temperature influences the rate of degradation of crude oil in the soil. At low temperatures, oil viscosity tends to increase, and the degradation of alkane decreases significantly as water solubility decreases. With increased temperature, between 30-40^{0}C and optimally within 30^{0}C at pH 7.5, crude oil is less viscous, dehydrogenases increase as degradation of crude oil is facilitated. At a temperature above 40^{0}C, the toxicity of crude oil may be experienced which adversely affects microbial activities and reduces the biodegradation rate [106-107]. Since *Pseudomonas Aeruginosa* is mesophilic, thus, tends to adapt and perform optimally at a temperature 30^{0}C [107]. However, a high concentration of crude oil is toxic to microbial growth and can lead to the death of microorganisms resulting in distortion of the bioremediation process. Hence, crude oil reduction is inversely proportional to the concentration [107], invariably, at a lower concentration of crude oil, *Pseudomonas Aeruginosa* will be more active as a result of a high rate of metabolism. The effect of concentration was evident in the study by Rahman *et al.* [107] which reported a decrease in biodegradation efficiencies, 70%, 67%, 63%, 52% as the oil concentration increases from 2.5%, 5%, 7.5% to 10% respectively.

The study by Karamalidis *et al.* [108] on the treatment of petroleum polluted soils involving stimulation of indigenous microorganisms and combined stimulation & inoculation with *Pseudomonas aeruginosa strain* showed varying degrees in hydrocarbon concentration in different treatments. The treatment of 191 days recorded for the indigenous cells; 94% of n-alkane degradation from 8025mg/kg to 481mg/kg at t=191d; total aliphatic fractions decreased by 89% on similar treatment period (from 17780mg/kg at t = 0d to 1951mg/kg at t = 191d). Also, combined stimulation and *Pseudomonas aeruginosa* inoculation showed a reduction in recalcitrant hydrocarbon fraction with 20% and 70% PAH biodegradation efficiency (after 35 and 150 days respectively) from the initial concentration of 58mg/kg to 17mg/kg at the end of the treatment. Degradation of n-alkanes by stimulated indigenous microbes recorded an increase in reduction efficiency with corresponding to an increase in time to attain 82 – 98% for n-C_{12} to n-C_{27} after 107 days and >82% for n-C_{28} to n-C_{34} after 191d. The overall biodegradation efficiency according to Karamalidis *et al.* [108] was observed to be 73.3%. It reported that bioremediation with free cells or encapsulated *Pseudomonas aeruginosa* has little effect on the treatment performance, but It will be noted that the introduction of *Pseudomonas aeruginosa* inoculum effectively reduces the treatment time and facilitate the removal of recalcitrant hydrocarbons [108].

Microbial biodegradation of resins fractionated from Arabian light crude was investigated by Venkateswaran and Harayama [109] using *P. aeruginosa* isolated from emulsified mixed population recorded 30% and 30% of resin and aromatics respectively from 5000ppm concentration of crude oil after 7 days of treatment. It reported an increased *P. aeruginosa* growth rate while degrading fractions of hydrocarbons. Similarly, the study by Mukherjee *et al.* [110] further buttresses the ability of *P. aeruginosa* to grow in oil-polluted soils as earlier reported by Venkateswaran and Harayama [109], while using selected fractions of hydrocarbons as a carbon source.

The degradation of hydrocarbon was investigated by Mukherjee *et al.* [111] using bacteria strains isolated from an oil field and cultured in a mineral media and hydrocarbon enrichment environment containing apt proportions of benzene, toluene, hexadecane, tributyrin, and glucose as an energy source for bacteria growth. The study observed, through bacteria identification that *P. aeruginosa* was the most versatile and popular bacteria strain in the isolate among other bacteria; *Acinetobacter spp. Flavobacterium multivorum* and *Flexibacter condensin* identified. The study reported the ability of *P. aeruginosa* strain PTZ-5 from the oil field to effectively utilize various fractions of hydrocarbons: hexadecane, benzene, and toluene as an energy source for growth while facilitating the remediation process of the hydrocarbons. The result reported the ability of *Acinetobacter calccoaceticus* ADPT to grow on hexadecane and not in alkane which suggests the inability of *Acinetobacter sp.* to perform in some hydrocarbon components [112]. In contrast to the performance of *Acinetobacter calccoaceticus* in the treatment, *P. aeruginosa* (PTZ-5) was tolerant of various hydrocarbon concentrations and showed great potential in the removal of hydrocarbons [110].

Tavassoli *et al.* [113] evaluated the degradation of asphaltene using microorganisms isolated from crude oil samples. Among the isolated and identified strains based on their morphological and biochemical characteristics is *Pseudomonas spp. TMU2-5, Bacillus licheniformis Tmu1-1,* *B. Lentus TMU5-2, Bacillus cereus TMU8-2, and Bacillusfirmus TMU6-2.* Biodegradation of asphaltene was highest with the mixed culture which recorded 48% removal efficiency when compared to pure cultures; *Pseudomonas spp.* with a 46% removal efficiency, degrading mostly branched alkanes, phenol, naphthalene, and acetone [111-112] and *Bacillus spp*. was effective in the degradation of benzene and PAH. It can be suggested that *P. aeruginosa* and *Bacillus spp.* can be effective in the clean-up of polluted sites by constant biodegradation of PHC [110]. Similarly, the asphaltene degradation was investigated by Pineda-Flores *et al.* [114] using of mixed culture of bacteria consortium with and without *P. aeruginosa* was investigated for the potency of each consortium. Biodegradation of asphaltene with a mixed culture (without *P. aeruginosa*) comprising of *Bacillus, Brevibacillus, Staphylococcus, and Corynebacterium* which uses asphaltene as energy source recorded of 46% after utilizing 8% of asphaltene in 13d with initial HC concentration of 5 g/L, at 25 ^{o}C for 60 days treatment period. However, the performance of 4 strains of bacteria containing *Pseudomonas, Citrobacter, Enterobacter, Staphylococcus and Lysinibacillus* recorded asphaltene removal efficiency of 11 – 51% both in shaking and static condition at 40 ^{o}C for 60 days study period [115-116]. Sequel to the mixed culture comparison, it can be inferred that the later, mixed culture containing *Pseudomonas* showed a slight improvement with regards to biodegradation efficiency under the same timeline. However, Honarmand *et al.* [116] recorded a higher asphaltene degradation rate more than Tavassoli *et al.* [113] and Pineda-Flores *et al.* [114], in the biodegradation of heavier fractions of crude oil using some bacteria strain, *Bacillus toyonensis BCT-7112*. The result of the study recorded asphaltene's reduction efficiencies of 64.85% and 60% at 25^{o}C and 45^{o}C respectively. The variation in the degradation efficiencies of different treatments may be attributed to concentration, operating temperature, and bioavailability of asphaltene for oil-degrading bacteria [106, 112].

The study by Rahman *et al.* [107] investigated the efficacy of crude oil remediation by a mixed consortium (containing *Micrococcus sp. GS2-22, Corynebacterium sp. GS5-66, Flavobacterium sp. DS5-73, Bacillus sp. DS6-8b and Pseudomonas sp.DS10-129* isolated from oil-polluted soil samples) and observed a decrease in biodegradation of crude oil as the concentration of oil increases. The result of the experiment recorded the highest removal efficiency of 78% with mixed consortium after 20 days period of incubation. However, for the single strain, *Pseudomonas sp. DS10-129* showed the highest degradation efficiency of 66% followed by *Bacillus sp. DS6-8b, Micrococcus sp. GS2-22, Corynebacterium sp. GS5-66, Flavobacterium sp. DS5-73* with 59%, 49%, 43%, and 41% respectively, as *Flavobacterium sp. DS5-73* recorded the lowest reduction efficiency. The appreciable biodegradation efficiencies recorded by these treatments are attributed to the reduction of the lag period required for the microorganisms to respond by the application of mixed or single cultured strain(s) of microorganism(s) [107].

Zhang *et al.* [117] investigated the degradation of n-alkanes and PAH in petroleum using *P. Aeruginosa DQ8* isolated from oil-polluted soil, cultivated in modified Basal Salt medium (BSM) for 5 weeks. The study showed that *P. aeruginosa DQ8* used oil as the energy source for its growth and degraded about 83±1.0% of 2%(v/v) diesel oil which includes C_{12}-C_{25} n-alkanes and other fractions with a total degradation of alkanes length greater than C_{20} and degradation efficiencies of 53.3±2.1%, 66.3±5.3%, and 46.6±3.4% for aromatic, nonhydrocarbons and asphaltenes, respectively. Similarly, Richard and Vogel [118] recorded a diesel oil reduction efficiency of 90% after 90 days of treatment using a sub-cultured bacteria consortium. Also, the study by Lin *et al.* [27] was in line with the prevailing trend, where effective degradation of phenanthrene (PHE) was attributed to P*seudomonas sp. BZ-3* isolated from crude oil-polluted soil, cultured in a mineral medium, and inoculated to different concentrations of PHE (500 mg, 1000 mg, 4000 mg) supplemented at 50 mg/L. It was observed that *Pseudomonas sp. BZ-3* utilized the PHE as an energy source [119] which is evident in the appreciable biodegradation efficiency of 75% of PHE (of 50 mg/L initial concentration) after 28 days of treatment. Further analysis of the result revealed that *Pseudomonas sp. BZ-3* degraded >45% PAH with two rings for non-aromatics, 36% of PAH in case of PHE, and Anthanthrene were recalcitrant to degradation as only 18% was degraded, PAH with 4 rings recorded 26% degradation efficiency in the case of pyrene. These are also agreed with results obtained by [119, 120] – which indicates that *Pseudomonas sp. BZ-3* possibly possesses an effective enzyme to foster the removal of PAHs.

The study by Varjani and Upasani [121] on the influence of activity parameters on the degradation of crude oil by *P. aeruginosa* NCIM-5514 noted that *P. aeruginosa* was feasible in remediation and enhancing commercial application on the surface and subsurface degradation of hydrocarbon in the polluted soils. The study considered the effect of environmental and nutritional conditions such as agitation, temperature, pH, NaCl concentration, petroleum, and non-petroleum energy source and its concentration, Nitrogen, and inoculum ratio on the growth of *P. aeruginosa NCIM-5514.* The result of the study showed that optimum growth of *P. Aeruginosa NCIM-5514* was observed at 1%(w/v) glucose at 180rpm with a temperature of 37^0C and pH 7.2, with 1%(w/v) inoculum for 4 days using crude oil and glycerol as an energy source. However, optimization of environmental parameters for the growth of *P. aeruginosa* affects the biodegradation ability and efficiency of the hydrocarbons by microbes [122] as good biodegradation can be feasible by adjusting some conditions while optimizing physical and chemical factors such as growth media, carbon source for effective biodegradation. The temperature and physicochemical nature of the contaminants affects the growth of *P. aeruginosa* [121].

In the biodegradation process, oxygen acts as a substrate in oxygenase catalysed reaction and doubles as an electron acceptor in oxic metabolism. Hydrocarbon is a good carbon source for *P. aeruginosa* growth, Priya and Usharani [123] signify that the type and C/N concentration used in the media culture is essential for the growth, biomass formation and biodegradation of hydrocarbon by *P. aeruginosa* [122]. Results showed the mesophilic, aerobic crude oil utilizer and halotolerant, nature of *P. aeruginosa* [121].

The study by Das and Mukherjee [124]] to evaluate the biodegradability of *Bacillus Substilis* and *P. aeruginosa* strain isolated from petroleum oil-polluted soil showed that *P. aeruginosa* was effective than *Bacillus Substilis* after 120 days of the experiment. Moreover, the two strains showed a significant decrease in the concentration of crude oil in the soil as compared to the control treatment. The study observed extensive growth and biosurfactant synthesis by exogenic microbes in oil-contaminated soils. The isolated bacteria were supplemented with 2%(v/v) petroleum and incubated at 45^0C and pH 7.0 for *P. Aeruginosa N and NM* strains [125] or at 55^0C temperature and pH 8.0 for *B. Subtilis* DM-04 strain [126] with 200rpm agitation. Conclusively from the study, *P. aeruginosa* showed a higher degradation efficiency of 75% against *B. Subtilis* with 53.6% representing a reduction from 84g/kg initial concentration to 21g/kg and 39g/kg respectively after 120 days of treatment. Also, a high level of crude oil degradation exhibited by *P. aeruginosa* in the study [124] due to significant breakdown and utilization of petroleum as a carbon source which invariably enhanced the growth of *P. Aeruginosa* as compared to *B.Substilis*. In contrast, Jackson and Pardue [127] and Hesnawi and Mogadami [128] inferred that the addition or introduction of nutrients into the treatment has minimal impact on the removal efficiency of the crude oil. However, the study by Shin *et al*. [129] and Atlas [130] concluded that microbial or organic amendment is essential since the indigenous microbial community is ineffective for optimum degradation of complex and recalcitrant hydrocarbons while Chaineau, Rougeux [131]] advocate for adequate nutrient addition with moderation) for a better TPH removal.

P. aeruginosa tends to decrease the surface tension of culture which suggests that strain might produce biosurfactants [117]. The production of surfactant may also contribute to the distribution and effective degradation of crude oil which promotes degradation of TPH with or without the addition of extra nutrients [132]]. Unlike other strains of bacteria, *P. Aeruginosa* can effectively degrade (n-alkanes and PAHs) [133] and different fractions of hydrocarbon which suggests its effectiveness for TPH degradation [107]. Besides, Zhang *et al.* [117] noted that *P. Aeruginosa* can use diesel oil and crude oil as a substantive energy source for growth while degrading the same effectively. Thus, the ability of *P. Aeruginosa* to degrade major components of crude oil inferred that it can be applied for the remediation of a vast group of petroleum fractions and remediation of crude oil contaminated soils. Table 2 shows the application of different bioremediation methods and their performances in hydrocarbon remediation.

Table 2. Bioremediation of crude oil contaminated Soils using different approaches

Bioremediation methods	Nutrient amendment	Contaminant	Degree of contamination/initial concentration	Process duration/ study period	Maximum removal efficiency	References
BAT	No amendment	Crude oil	47.28mg/g	98 days	22.40%; 32.1%	[21]
BAT	No amendment	Spent motor oil	9830mg/kg – 14439mg/kg	10 weeks	50%	[67]
BAT	No amendment	Petroleum	3g/kg, 30g/kg and 60g/kg oil conc./kg of soil	90 days	13.01 – 15.33%	[77]
BST	Aged Refuse	Petroleum	47.28mg/g	98 days	74.64 – 89.83%	[21]
BST	Slurry + land farming	Crude oil	20454ppm/ha	2 months	88%	[41]
BST	Land farming	Crude oil		15 months	80%	[53]
BST	Sewage Sludge	Crude oil	5kg/l	9 weeks	45 – 65.6%	[34]
BST	Sewage sludge	Crude oil	1000mg/kg	10 weeks	45% - 60%	[35]
BST	Organic waste; (goat manure, poultry droppings, and cow dung)	Crude oil	53966.60mg/kg	28 days	70.7 – 87.1%	[37]
BST	Composted municipal waste	Petroleum	Diesel fuel: 16000±83mg/kg Spent engine oil: 18333±97mg/kg; Crude oil: 23000±101mg/kg	15 days	40 – 75.8%	[55]
BST	Poultry manure	Crude oil	3666mg/g	157 days	96.01%	[134]
BST	Organic fertilizer	Crude oil	4000mg/kg	155 days	90.01 - 92%	[135]
BST	Organic (cow dung, (CD); palm kernel husk ash, (PKHA) and inorganic fertilizer (NPK)	Crude oil	Varying degrees 2%, 4% and 6% to 1000g soil	40 days	84.62% - (CD + NPK); 76.80% - NPK	[38]
BST	Organic waste (refuse)	Crude oil	42mg/g	96 days	44 – 87%	[136]
BST	Organic (poultry droppings, and goat dung) and inorganic fertilizer (NPK) and sawdust	Crude oil	20L of crude oil per 3kg soil	112 days	60.7% - 88%	[36]
BST	Plant and animal organic and NPK	Crude oil	200g crude oil per 1kg soil	8 weeks	89 – 96.89%	[137]
BST	Organic (poultry manure) and inorganic (NPK) fertilizer	Crude oil	300mg/kg	12 weeks	76.42 – 86.97%	[47]
BST	Chicken manure and	Crude oil	28.8mg/kg to 70.27mg/kg	42 days	>60%	[138]
BST	Sheep waste and goat waste compost	Petroleum hydrocarbon	NA	28 days	pH reduced from 6.63 to 8.22	[50]
BST	Cow dung	Crude oil		28 days	89 – 98%	[45]
BST	Goat dung	Crude oil	130g/l	56 days	70 – 76%	[42]
BST	Animal Waste (poultry manure, piggery manure, goat manure and chemical fertilizer)	PHC mixture (Kerosene, diesel and gasoline mixture)	1kg soil with 10%(w/w) PHC	4 weeks	Poultry manure: 73%; Piggery manure: 63%; Goat manure: 50%; NPK: 39%	[139]
BST	*Capra aegagrus hircus*; Goat manure	Crude oil	50ml/kg	14 days	62.08%	[52]
BAU	Indigenous microbes, and free cells of *P. aeruginosa*	Petroleum	8025mg/kg; 17780mg/kg	191 days	n – alkane – 94%; Aliphatic – 89% respectively	[108]

Bioremediation methods	Nutrient amendment	Contaminant	Degree of contamination/initial concentration	Process duration/ study period	Maximum removal efficiency	References
BAU	*Acinetobacter sp. T4* and SM8 consortium	Crude oil	5000mg/L	28 days	19 – 34% and 12 – 20%	[69]
BAU	*P. aeruginosa*	Crude oil	5000ppm	7 days	30 % for resin and aromatics	[109]
BAU	*Pseudomonas spp. TMU2-5, Bacillus licheniformis Tmu1-1, B. Lentus TMU5-2, Bacillus cereus TMU8-2 and Bacillus firmus TMU6-2.*	Asphaltene	-	60 days	48% for Mixed culture and 46% for *Pseudomonas sp.*	[113]
BAU	*Bacillus toyonensis BCT-7112*	Asphaltene	5g/L	50 days	64.8% and 60% at 25ºC and 45ºC respectively	[140]
BAU	a mixed culture comprising *P. aeruginosa, Bacillus, Brevibacillus, Staphylococcus and Corynebacterium*	Asphaltene	5g/L	13 days	46%	[114]
BAU	by mixed consortium containing *Pseudomonas sp.DS10-129, Bacillus sp. DS6-8b, Micrococcus sp. GS2-22, Corynebacterium sp. GS5-66, Flavobacterium sp. DS5-73*	Crude oil	1% - 10% crude oil concentration	20 days	78% for mixed consortium and 66%, 59%, 49%, 43%, and 41% for single strain respectively,	[107]
BAU	*P. Aeruginosa DQ8*	Diesel oil	2% *vlv*	5 weeks	53.3±2.1%, 66.3±5.3%, and 46.6±3.4% for aromatic, non-hydrocarbons and asphaltenes respectively	[117]
BAU	*Pseudomonas sp. BZ-3*	PAH	50mg/L	28 days	>45% PAH with two rings for non-aromatics, 36% of PAH in case of PHE and Anthanthrene	[27]
BAU	*B. Subtilis* DM-04 strain and *P. Aeruginosa N and NM* strains	Petroleum	2%(v/v) petroleum (84g/kg)	120 days	75% for *P. Aeruginosa* and *B. Subtilis* with 53.6%	[124]
BAU	Bacteria consortium	PAH	936.1mcg/kg	90 days	3-ring PAH: 18.7-35.2%; 4-ring PAH: 21.8-33.2%; 5-ring PAH: 17.3 – 40.5%	[68]
BAU	Bacteria consortium	Crude oil	3, 30 and 60 g	90 days	73.89, 73.76 and 58.31% respectively	[77]
BST & BAU	Commercial NPK Fertilizer, Humic Substances (HS), Organic industrial waste (NOGRO) and yeast bacteria consortium	Polyaromatic hydrocarbon (PAHs)	63.0g TPH/kg Soil	90 days	46 – 64%	[70]
BST & BAU	Yeast and nutrient amendment	TPH	16300mg/kg	180 days	83% - 96%	[74]

Bioremediation methods	Nutrient amendment	Contaminant	Degree of contamination/initial concentration	Process duration/ study period	Maximum removal efficiency	References
BST & BAU	Microbial inoculation and nutrient supplement	Crude oil	277.5g Crude oil per 1850g soil	156 days	56 - 77%	[64]
BST & BAU	Nutrient supplement and mycobacterium	Pyrene (PAHs)	700mg pyrene per 1kg soil	2 weeks	84.29% (BST + BAU); 57.86% - BAU; 50% - BST	[65]
BST & BAU	Inorganic fertilizer and dihydrogen orthophosphate and consortium of bacteria	Spent motor oil	9830mg/kg – 14439mg/kg	10 weeks	75% (BST); and 66% (BAU)	[67]
BST and BAU	Bacteria consortium and nutrient supplement	Petroleum	3g/kg, 30g/kg and 60g/kg oil conc./kg of soil	90 days	43.02 – 58.36% (BST); 58.31% - 73.89%;(BAU)	[77]
BST & BAU	Microbial consortium and nutrient	Crude oil	3000bbl/acre	70 days	97%	[141]
BVT & composting technology	Organic fertilizer as a stimulant	Crude oil	$7.0X10^4$mg/kg	40 days	45%	[80]
BVT	Organic nutrient and moisture O_2	Hydrocarbon (light and heavy - PAHs)	4900ppm TPH	70 days	62% - 98%	[81]
BVT	Nutrient addition + oil-degrading bacteria and O_2	Diesel oil	2000mg/kg	112 days	96%	[82]
BVT & biosparging and phytoextraction	Air	Crude oil and heavy metal	NA	40 – 50 days	60% of c/o (BV and BS); 50% of metals (phytoextraction)	[85]
BVT & BST & BAU	Brewery waste (BW)	Diesel oil	1kg soil with 10% (w/w) diesel oil	28 days	BVT + BW: 91.5%; BST + BAU: 78.7%; BVT: 61.7%	[92]
BVT	Oxygen	Diesel oil	8000mg/kg	5 months	Increase TPH removal	[87]
BVT	Oxygen	Crude oil	40g/kg	120 days	85%	[88]
BVT	Oxygen	Phenanthrene	1000mg/kg	7 months	93%	[79]
BVT & biotrickling filter technology	Microbial inoculum	Crude oil	14mg/g	121 days	90 - 99%	[90]

BST - Biostimulation; BAU – Bioaugmentation; BVT – Bioventing; BAT: Bioattenuation

4. Challenges and prospects

Bioremediation has the potential to be the most eco-friendly and long-term solution for degrading various pollutants from contaminated soils and sediments. However, the lack of appropriate nutrient may slow down or hinder the bioremediation process as synergies between soil, nutrients, and microorganisms facilitate pollutant degradation by contributing to decontamination, increase in growth, co-metabolism, and the excessive expression of catabolic genes. Hence, the effectiveness of biostimulation depends on the introduction of an appropriate amount of nutrients to the polluted site to promote the activities of native organisms. Since microbes are pervasive, it is obvious that contaminant degraders are normally found in the contaminated area, and their quantities and metabolism may fluctuate in relation to contaminant toxicity and nutrient availability; thus, the application of organic, inorganic, or agroindustrial wastes with suitable nutrient constituents, particularly NPK will aid in overcoming the problem of lack of substrates in most contaminated sites. Moreover, it was reported that excess nutrients hinder metabolism and microbial diversity [142].

Bioaugmentation is a key strategy for incorporating or expanding microbial populations with degradative abilities. The microbial consortium has been shown to break down contaminants more effectively than pure isolates. This can be attributed to individual isolates' metabolic diversities, which may stem from their isolation source, adaptative mechanism, or contaminant components, and will result in interactive effects, for an effective contaminant degradation when such isolates are mixed [143]. However, some hydrocarbon fractions are recalcitrant to microbial degradation. While bioaugmentation is successful, conflict among endogenous and exogenous microorganisms, the possibility of incorporating pathogenic species into an ecosystem, and the likelihood that introduced microbes will not thrive in the new site make bioaugmentation a precarious method. The application of carrier materials (agar, agarose, gelatin etc) will aid in the mitigation of some of these issues [144].

Bioventing technology is an aerobic process of requires the injection of regulated quantities of air or oxygen directly or indirectly into the vadose zone of the contaminated site to facilitate contaminant degradation. The use of pure oxygen has proven to be more effective in the bioventing process due to its ability to effectively ventilate the vadose to enhance the mineralization of TPH present in the contaminated soil. This is more efficient than the use of air sparged (injection of pressurized air) or hydrogen peroxide (H_2O_2) as oxygen (O_2) source delivered a lower amount of dissolved O_2 compared to the direct injection of O_2 [145]. Several studies have shown that the use of H_2O to supply oxygen to the subsurface zone is less effective due to its low penetration power when compared to gas (air or O_2). Also, hydrogen peroxide has been criticized for a similar reason, since its low penetration power restricts the supply of oxygen to the vadose zone where it is required for microbial activities for effective bioremediation [78]. Consequently, Huling *et al.* [146] found that increased concentration of H_2O_2 up to 100mg/L may have an inhibitory effect on biodegradation efficiency, and the stability and toxicity of H_2O_2 depend on pollutant components, site type, and other environmental conditions. However, the direct application of oxygen in a bioventing system is efficient but not economically viable due to high operation cost but the use of atmospheric air is cost-effective and have shown promising potentials for industrial-scale application [88].

With the application of biosurfactants, the indigenous microbes at contaminated sites will possibly degrade contaminant faster than allochthonous microbes under ideal environmental parameters. Biosurfactants tend to trigger desorption and solubilization of contaminants, thereby raising mass transfer, to boost pollutant accessibility to biodegrading organisms, particularly crude oil contaminated sites [147]. Rhamnolipids are the best-studied kind of glycolipid when it comes to biosurfactant. The optimization of rhamnolipid production from a *P. aeruginosa* S2 strain in different media source (containing C & N introduced as glucose and NH_4NO_3 respectively) showed that the best C/N ratio for rhamnolipid yield was around 11:4 [148]. However, improving the media and culture landscape is not the only thing that needs to be changed to enhance production. In this regard, overproducing mutant or recombinant strains have been developed and used to increase biosurfactant yield [149]. Various perspectives have also been adopted to mitigate the complex environmental control of rhamnolipid biosynthesis, as well as to substitute the opportunistic pathogen, *P. aeruginosa* with a healthy industrial strain. Although hyper-producing strains have been shown to improve biosurfactant yield, large scale production of recombinant hyper-producing strains has yet to be extensively explored, hence, the production of biosurfactants is still in its initial phases.

Due to the eco-friendly and biodegradable properties, microbial surfactants are favoured over chemical counterparts and the current focus of research is on the industrial-scale production of these compounds. The large-scale implementation of biosurfactants to contaminated sites, however, is not economically viable because of high operating costs and poor scalability. Biosurfactant yield could be increased by using both industrial and agricultural wastes as substrates sources for presumed biosurfactant producers during fermentation. For optimal production of glycolipids, lipopeptide, emulsan, alasan, and biodispersan, a comprehensive evaluation is considered necessary to attain the intended production limit. Several factors such as media compositions, precursors, and genetic framework must be considered for effective biosurfactant development. These factors are important and influence the biosurfactant development process as well as the biosurfactant's final quantity and consistency [149]. As a result, further research is required in this field to boost yield production while also looking for new forms of biosurfactants to use in hydrocarbon bioremediation processes.

However, increased soil salinity can inhibit the application of bioremediation methods by limiting the microbial components' growth [150]. This can be mitigated by focusing and incorporating halophytes and salinity-resistant bacteria. Due to the importance of the halophyte or soil microorganisms contribution in bioremediation of crude oil-polluted saline soils, finding microorganism species that can acquire biomass amidst salinity and crude oil, should be prioritized. Also, the integration of recombinant microbes through molecular approaches including "omics technologies" (genomics, metabolomics, proteomics, and transcriptomics) can greatly contribute to the development of new, genetically engineered contaminant remedial approaches which can help mitigate these challenges. This technology can allow the identification of vital metabolites and aspects of pollutant degradation pathways for successful bioremediation. However, the universal acceptability of this approach remains a drawback to its implementation.

5. Conclusion

Bioremediation methods are discrete and have proven successful in cleaning, maintaining, and reviving sites contaminated with crude oil, as evidenced by the appreciable biodegradation efficiencies recorded. Microbes play important role in bioremediation; hence, their biodiversity, plethora, and population composition in polluted sites offer insight into the outcome of any bioremediation process, if all external factors that can obstruct metabolic processes are kept within acceptable limits. The biodegradability of undesirable toxic wastes is affected by competition within biological agents (such as fungi, bacteria, and algae), deficient supply of required substrates, abiotic factors (aeration, moisture, pH, temperature), and decreased bioavailability of contaminants. Bioremediation is dependent but not limited to various factors, such as cost, site characteristics, pollutant form and concentration. The main step toward effective bioremediation is site characterization, which aids in the development of the most appropriate and effective bioremediation technique. Besides, considering the significance of bacterial transmission quality and capability in this context, future research should investigate different microbial carriers with the potential to enhance the physicochemical properties of soils for an effective clean-up. Also, broadening areas of theoretical research, improving relevant molecular genetic engineering technologies, tracking, and performing risk assessments of environmental contaminants, and developing the framework on legislation and guidelines for environmental sustainability should receive further focus in future studies for sustainable development of bioremediation technology.

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To whom correspondence should be addressed: professor Yusuf Makarfi Isa, School of of Chemical and Metallurgical Engineering, University of the Witwatersrand, Johannesburg 2050, South Africa;
e-mail: yusuf.isa@wits.ac.za