Chapter

Biological Treatments for Petroleum Hydrocarbon Pollutions: The Eco-Friendly Technologies

Innocent Chukwunonso Ossai, Fauziah Shahul Hamid and Auwalu Hassan

Abstract

Anthropogenic activities introduce petroleum hydrocarbons into the environments, and the remediation of the polluted environments using conventional physicochemical, thermal, and electromagnetic technologies is a challenging task, laborious work, and expensive. The ecotoxicological effects and human health hazards posed by petroleum hydrocarbon pollutions gave rise to the call for “green technologies” to remove petroleum hydrocarbon contaminants from polluted environments. It is imperative to transition from the conventional physicochemical treatments methods that are expensive to more eco-friendly biological treatment technologies that reduce energy consumption, chemicals usage, cost of implementation and enables more sustainable risk-based approaches towards environmental reclamation. The chapter summarises and gives an overview of the various biological treatment technologies adapted to the remediation of hazardous petroleum hydrocarbon polluted sites. Biological treatment technologies include; bioremediation, biostimulation, bioaugmentation, bioattenuation, bioventing, biosparging, bioslurry, biopiling, biotransformation, landfarming, composting, windrow, vermiremediation, phytoremediation, mycoremediation, phycoremediation, electrobioremediation, nanoremediation, and trichoremediation. They are green technology approaches widely adopted, scientifically defensible, sustainable, non-invasive, ecofriendly, and cost-efficient in the remediation of petroleum hydrocarbons polluted environments compared to the physicochemical, thermal, and electromagnetic treatments technologies, which are rather destructive and expensive. The chapter provides detailed illustrations representing the various biological treatment technologies for a comprehensive understanding and successful implementation with their subsequent benefits and constraints.

Keywords: bioremediation, phytoremediation, phycoremediation, mycoremediation, vermiremediation, trichoremediation

1. Introduction

The intensive development of human civilisation, urbanisation, population growth, economic development, and impulsive industrialisation have expanded
petroleum hydrocarbon production, distribution, and utilisation. This phenomenon caused a gradual depletion of natural petroleum reserves and increasing demand for petroleum products [1]. The petroleum industry is one of the world’s largest and most important global industries with a primary function in oil and gas production [2]. The global economy has become entangled with infrastructure that depends on petroleum hydrocarbon products such as petrol, diesel, kerosene, jet fuel, fuel oil and motor oils [3]. These products have become the main source of primary energy globally. Their exploration has transformed the world by providing fuel and raw materials for various industries for various applications and serving as feedstock for several consumer goods, thus playing an increasing and relevant role in our daily lives [4]. Apart from the benefit of being an important energy source, the products have caused the environment to become constantly bombarded with hazardous pollutants [5]. The causes of the pollutants entering the environment are diverse (Figure 1) as the amount of individual petroleum hydrocarbon components are significantly substantial. Pollution caused by petroleum hydrocarbon products poses direct and indirect ecotoxicological effects and human health risks [6–8].

The environmental fate and toxicokinetics of petroleum hydrocarbons are critical aspects of risk assessment because they determine human or environmental receptor exposure to pollution [9, 10]. When discharged or released in the environment, the components of petroleum hydrocarbons undergo weathering processes [11], involving various processes such as adsorption, volatilisation, dissolution, biotransformation, photolysis, oxidation, hydrolysis through interaction with microorganisms and metabolic pathways [12, 13]. The level at which various components of petroleum hydrocarbon deteriorate under weathering processes depends mainly on the nature of the petroleum hydrocarbon compounds, composition, physical and chemical characteristics [14]. A wide variety of natural processes involved in the fate and behaviour of petroleum hydrocarbons in the soil are illustrated in Figure 2. The weathering process includes adsorption to soil particles and organic materials, volatilisation to the atmosphere [15], and dissolution in water [16]. Environmental conditions, such as temperature, humidity

![Figure 1. Sources of petroleum hydrocarbon pollution.](image-url)
and precipitation, affect the weathering process [11]. The aliphatic hydrocarbons are more readily biodegraded than aromatic hydrocarbons [17], and the aliphatic hydrocarbons are more volatile because of their molecular nature [18]. If volatilisation is the primary weathering process, the loss of lower molecular weight aliphatic hydrocarbons is the most dominant change in the petroleum hydrocarbon, which may be the principal air pollutants causing air pollution at contaminated sites [19]. Volatilisation changes the residual non-aqueous liquid (NAL), affecting its transportation over time [20]. The petroleum hydrocarbon vapours are transported to the gaseous phase through diffusion or advection, and the process depends on the soil pore characteristics [21]. The gas-phase mass transfer in a polluted soil consists of volatilisation from the non-aqueous phase liquid (NAPL) and partitioning in gaseous/aqueous interphase [14].

However, considering the environmental impacts of petroleum hydrocarbons which affect the surface soil, subsoil, sediments, surface water and groundwater coupled with the human health risk. It has become imperative to transition from conventional treatment technologies such as physicochemical treatments, thermal/heat treatments, electric and electromagnetic treatments, acoustic and ultrasonic treatments that are challenging, laborious, extensive and expensive to more feasible biological treatment technologies that are sustainable, eco-friendly and economical.

2. Biological treatment technologies

Biological treatment technologies that have shown remarkable success for in situ and ex situ remediation of petroleum hydrocarbons are illustrated in Figure 3.
The feasibility of the biological treatment technology depends mainly on the limiting factors and the location of the contaminants. Treatability also depends on the soil, sediments, surface water, and groundwater properties, whether it is localised or removed, excavated, and transported for treatment at an off-site treatment facility. If treatment is on-site, the term *in situ* suffices, and if treatment is off-site, *ex situ* suffices [22]. The biological treatment technologies can remediate or degrade petroleum hydrocarbons and various organic contaminants to simpler and non-toxic substances without any long-term adverse effect on the impacted environments [23]. The general advantage of biological treatment technologies is that treatments do not disrupt the environment. The general constraint is that treatments usually require a long treatment period ranging from months to several years for a satisfactory and effective removal of contaminants. High concentrations of contaminants may result in low microbial activity with low or insufficient removal efficiency [24].

### 2.1 Bioremediation

Bioremediation is an eco-friendly, sustainable, and cost-effective means of restoring and cleaning soil contaminants such as petroleum hydrocarbons in polluted environments. The technique comprises the natural degradation of petroleum hydrocarbon contaminants by petroleum hydrocarbon-degrading microorganisms such as bacteria, fungi, yeasts, and algae. Bioremediation removes and neutralises hazardous petroleum hydrocarbon contaminants to non-toxic or simpler compounds.
such as carbon (IV) oxide and water through oxidation process under aerobic conditions by the microorganisms with the nutrient provision and optimisation of the constraining factors for efficient metabolic activities [25, 26]. The petroleum hydrocarbon-degrading microorganisms in the soil participate in defining the metabolic pathways and mechanisms of the microbial degradation of petroleum hydrocarbons [27]. Bioremediation of alkanes typically occurs via a sequential oxidation process by a few microbial enzymes (i.e., alkane monooxygenases or cytochrome P450 oxidases, alcohol dehydrogenases, and aldehyde dehydrogenases) and connects to the cytosolic fatty acid metabolism (Figure 4).

Some genes affiliated with the outset of petroleum hydrocarbon metabolism have been identified, as alkB (encoding alkane monooxygenase) and ndo (encoding naphthalene dioxygenase). These genes are activated under aerobic conditions to degrade alkanes and polycyclic aromatic hydrocarbons (PAHs), respectively [28]. Before implementing the bioremediation, it is essential to consider all the limiting factors such as energy sources, pH, temperature, nutrients and inhibitory substances, which may affect the success of the bioremediation process [29]. In bioremediation, the aliphatic petroleum hydrocarbons are more amendable or degradable by the microorganisms than the long-chain and the branched or cyclic chain petroleum hydrocarbons [19]. The petroleum hydrocarbon-degrading microorganisms utilise carbon compounds as energy sources, growth, and reproduction [30]. Bioremediation using selected microorganisms or genetically modified microorganisms is increasing the interest of many researchers.

Some of the most commonly isolated petroleum hydrocarbon-degrading bacteria belong to the genus Acinetobacter, Alcaligenes, Paenibacillus, and Pseudomonas [31] and are recognised to efficiently degrade hazardous petroleum hydrocarbon contaminants into simpler compounds [32, 33]. In addition, fungi species such as Penicillium, Fusarium, and Rhizopus have been isolated and utilised in the bioremediation of petroleum hydrocarbon contaminated soil and sediments [34, 35]. However, bioremediation of petroleum hydrocarbon has been in use since 1940 but gained popularity after the Exxon Valdez spill in 1980 [36]. Bioremediation has been successfully

![Figure 4. Microbial bioremediation of petroleum hydrocarbon [27].](image-url)
applied worldwide in environmental oil pollution mitigation, such as in the oil spills in Prince William Sound, Alaska, in 1989 [37] and the Gulf of Mexico in 2010 [38], and it is a promising strategy for environmental cleanup in contaminated mangrove sediments [28, 39].

The advantages of bioremediation include; minimal disruption of the ecosystem, permanent elimination of contaminants, cheap operation costs, and can be coupled with other treatment technologies. The disadvantages include extensive monitoring, production of unknown by-products, long duration to complete bioremediation, and bioremediation limited to biodegradable compounds [40].

2.2 Biostimulation

Biostimulation involves adding stimulatory materials, organic wastes (Figure 5), bulking agents, nutrients amendments, bio-surfactants, biopolymers, and slow-release fertilisers to enhance and support microbial growth and enzymatic activities of the indigenous microorganisms in the contaminated soil for remediation activities [23, 41, 42].

Biostimulation occurs by optimising various rate-limiting parameters such as pH, temperature, aeration, macromineral nutrients, and electron acceptors such as carbon, oxygen, nitrogen, phosphorus, and potassium, which accelerate the metabolic activities of the indigenous microorganisms [43]. Biostimulation can be performed in situ and ex situ but depends on the existence of the indigenous microorganisms with the capacity to degrade the hazardous contaminants [44, 45]. The microbial community composition becomes evener and richer during biostimulation [46], and the requirements include the presence of correct microorganisms, ability to stimulate target microorganisms, ability to deliver nutrients, C:N:P-30:5:1 for balance growth [45]. A study conducted by Singh et al. [47] investigated biostimulation of petroleum hydrocarbon contaminated soil using bacterial consortia and nutrient mixture to achieve a TPH removal efficiency of 99.9% after 18 months.

The benefits of biostimulation include; the use of native microorganisms adapted to the environment, being eco-friendly and cost-effective, preventing ecosystem disturbance, and can be coupled with other treatment technologies. The disadvantages include; it depends on environmental factors that control the potentiality, requiring extensive monitoring and scientific observations, contaminants may be non-biodegradable after adsorption to soil particles, and it takes a long duration to complete degradation [48, 49].

Various organic wastes have been used for biostimulation to optimise the degradation and removal of total petroleum hydrocarbons in the polluted soil [50–52].

2.3 Bioaugmentation

Bioaugmentation involves adding exogenous microbial cultures, autochthonous microbial communities, or genetically engineered microbes with a specific catabolic activity that have adapted and proven to degrade contaminants to enhance degradation or increase the rate of degradation of contaminants [17, 53–55]. Alexander [56] described bioaugmentation as inoculating contaminated soil or sediments with specific strains or consortia of microorganisms to degrade pollutants in the soil. Soil microbial community composition changes while microbial diversity decreases by bioaugmentation treatment [46].
Genetically engineered microorganisms have shown potential in bioaugmentation, exhibiting enhanced degrading capabilities for broad coverage of chemical and physical pollutants [57]. In the oil-polluted site of ONGC field in Gujarat, India, Varjani et al. [58] demonstrated *in situ* bioaugmentation using hydrocarbon utilising
bacteria consortium comprising six bacterial isolates for degradation of petroleum hydrocarbon contaminants and achieved removal efficiency of 83.7% in 75 days. Corvino et al. [59] also demonstrated bioaugmentation by using autochthonous fungi from petroleum hydrocarbon contaminated soil to degrade clay soil contaminated with petroleum hydrocarbons and achieve a removal efficiency of 79.7% after 60 days period.

The benefits of bioaugmentation include; less labour demand, the microbes do the work once introduced, microbial strains, mixed cultured or indigenous microbes can be used, eco-friendly and cost-effective, and can be carried out *in situ* without soil excavation. It can be combined with other treatment technologies. The disadvantages of bioaugmentation include; microbes require an appropriate environmental condition to thrive, the microbes may not metabolise all the contaminants completely, indigenous microbes may outcompete the introduced microbes, long duration to complete the remediation and may require genetically engineered microbes for degradation of contaminants [60].

2.4 Bioattenuation

Bioattenuation or natural attenuation is the use of naturally occurring processes, including a variety of physical and biochemical processes without human intervention, to remove, transform, neutralise and reduce the mass, volume, concentration, and toxicity of hazardous contaminants such as petroleum hydrocarbons in the environment by the activities of the indigenous microorganisms [28]. The process occurs through advection, dispersion, sorption, dissolution, volatilisation, chemical transformation, abiotic and biological transformation, stabilisation, and biodegradation [42]. Bioattenuation is applicable for contaminated environments with low contaminant concentrations and used in places where other remediation methods cannot be adopted [61].

The benefits of bioattenuation include; it can be adopted in all areas, causes minimal disruption of the site and the environment, low cleanup cost and can be used in conjunction with or as a follow up to other remediation methods. The disadvantages include; it is not all contaminants that are susceptible to rapid and complete degradation, it requires extensive site monitoring over a long period, it is limited to biodegradable contaminants, it depends on environmental factors that control potentiality for its success, and bioattenuation alone is inadequate and protracted in many cases [62].

2.5 Bioventing

Bioventing is an *in situ* bioremediation technology that utilises the indigenous microorganisms to biodegrade hazardous organic pollutants adsorbed to the soil. The technique involves injecting air (oxygen) into the contaminated soil to increase the *in situ* degradation and minimise the emission of volatile contaminants to the atmosphere [63, 64]. The injection of air into the soil stimulates and increases aerobic conditions for the growth of indigenous microorganisms and enhances the catabolic activity of the contaminants [65]. The mechanism of the bioventing process is similar to soil vapour extraction. Soil vapour extraction removes volatile pollutants through volatilisation, while bioventing systems promote biodegradation and minimise volatilisation [66]. Bioventing is helpful in the remediation of petroleum hydrocarbon contaminated soil. A bioventing layout using extraction vent wells is illustrated in Figure 6.
In a bioventing system study conducted by Agarry and Latinwo [42], the bioventing process was demonstrated on diesel oil-contaminated soil amended with brewery effluents as an organic nutrient source and achieved a removal efficiency of 91.5% over 28 days period. A similar study by Thomé et al. [68] also assessed the bioventing process on diesel-contaminated soil without any soil amendment and obtained a removal efficiency of 85% after 60 days.

The benefits of bioventing include; it can be deployed for in situ, and ex situ cleanup of contaminants, causes minimal disruption of the environment, low cleanup cost, and can be used in conjunction with other treatment technologies or as a follow up to other remediation methods. The disadvantages include; it does not promote remediation when the contamination zone is anaerobic, difficult to minimise environment release, low permeability soil pose a challenge due to its limited ability to distribute air through the surface, lab-scale and pilot-scale cannot guarantee treatment standards for specific contaminants of concern. Bioventing alone is inadequate and protracted in many cases [69].

2.6 Biotransformation

Biotransformation is a biotechnological process that involves modifications in the chemical constituents of the hazardous pollutants by the microorganisms or enzyme-mediated systems to form molecules with high polarity [70]. The mechanism transforms organic compounds from one form to another to reduce the contaminants’ toxicity and persistence [71, 72]. Naturally, the biotransformation process occurs very slowly and is nonspecific and less productive. But microbial biotransformation or biotechnology generates high amounts of metabolites, more rapid and productive outcomes, with more specificity. Microbial biotransformation helps modify and transform various contaminants and a large variety of compounds, including petroleum hydrocarbons in the soil [69]. Biotransformation of petroleum hydrocarbon
Hazardous waste management involves the management of hazardous materials that are harmful to human health or the environment. Contaminated soil occurs through bacteria, fungi, and yeast metabolic activities [38]. However, genetically modified organisms (GMOs) or genetically engineered microorganisms (GEMs) have shown potential in the biotransformation of contaminants in soil [57]. Biotransformation processes occur through oxidation, reduction, denitrification, condensations, isomerisation, hydrolysis, sulphidogenesis, methanogenesis, functional group introduction, and new bonds, as illustrated in Figure 7 [73].

In a pilot-scale investigation, Al-Bashir et al. [74] demonstrated a biotransformation study of naphthalene at the concentration of 50 mg/L in a slurry system under denitrifying conditions for 50 days. The results indicated that 90% of the total naphthalene was transformed after 50 days at a maximum mineralisation rate of 1.3 mg L$^{-1}$ per day.

The benefits of biotransformation include; it can be deployed for in situ and ex situ cleanup processes, uses microbial enzymes to metabolise contaminants and causes less disruption of the site and the environment. The disadvantages include; it may constitute cost due biotechnological process to synthesise biocatalysts, biosurfactants and enzymes, the contaminants may inhibit or kill the microbes, efficiency depends on the quality of the biocatalysts produced by microbes, required extensive biomonitoring and assessment, and required modification of microbes to produce target biocatalysts [69].

2.7 Biosparging

Biosparging involves the injection of air (oxygen) and nutrients into the saturated zone under pressure to increase groundwater oxygen concentration to stimulate biological activities of the indigenous microorganisms to degrade contaminants [67, 75].

![Figure 7. Biotransformation mechanism under the denitrifying conditions.](image)
Biosparging technology helps to reduce the contaminant concentration adsorbed to the soil, within the capillary fringe above the water table, and contaminants dissolved in the groundwater. The effectiveness of biosparging depends on soil permeability and pollutant degradability [76]. **Figure 8** illustrates the biosparging process in a polluted site.

In a study conducted by Kao et al. [78], a biosparging technique was deployed in a petroleum oil spill site for 10 months, and the result produced 70% removal efficiency for benzene, toluene, ethylbenzene and xylene (BTEX) within the remedial period.

The benefits of biosparging include; the equipment is easy to install, creates minimal disturbance to site operation, requires no soil removal or excavation, and a low air injection rate minimises the potential need for vapour capture, and treatment is cost-competitive. The limitation of biosparging is in predicting the direction of airflow in the process as it depends on the high airflow rate to achieve pollutant volatilisation and promote degradation [79]. It is site-specific and can cause the migration of contaminants, some interactions among complex chemicals and biophysical processes are not well understood and used only where suitable [66].

### 2.8 Bioslurry

Bioslurry involves the treatment of contaminated soil in a controlled bioreactor such as sequencing batch, feed-batch, continuous and multistage bioreactors [80, 81]. In a bioslurry treatment system, nutrients are added to enhance microbial activities to degrade hazardous contaminants. The bioslurry reactor is designed with various process controls to monitor, control, and manipulate temperature, mix, and add nutrients to achieve maximum removal efficiency. Amendments such as designer bacteria, surfactants, and enzyme inducers can be used in slurry bioreactors to stimulate and enhance biodegradative activities [82]. Bioslurry reactors may be constructed to provide sequential anaerobic/aerobic treatment conditions, as illustrated in **Figure 9**.

Bioslurry is an *ex situ* technology that can be used for bioremediation of problematic sites (when the less expensive natural attenuation or stimulated *in situ* bioremediation are not feasible [84]. The technology has been applied only to remove substances that are not readily degradable and non-halogenated volatile organic compounds, petroleum hydrocarbons and explosive compounds. Slurry-phase bioreactors

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**Figure 8.**
*Biosparging in petroleum hydrocarbon polluted soil [77].*
containing co-metabolites and specially adapted microorganisms are used *ex-situ* to treat halogenated compounds, pesticides, polychlorinated biphenyls (PCBs) [85].

In a study conducted by Tuhuloula et al. [86, 87], bioslurry treatment was demonstrated on petroleum hydrocarbon contaminated soil obtained from the oil drilling site of Pertamina Petrochina in Indonesia using microbial consortia of *Bacillus cereus* and *Pseudomonas putida*. The result obtained showed naphthalene removal efficiency between 79.35–99.73% in a slurry bioreactor after 49 days. A similar pilot-scale study conducted by Zhang et al. [85] evaluated aerobic bioslurry phase reactors in treating soil contaminated with explosive compounds (2,4 and 2,6-dinitrotoluenes) at Army Ammunition Plant in Tennesse and Wisconsin, USA. The result obtained showed a removal efficiency of 99%.

The benefits of bioslurry-phase treatment include increased intimated contact between microorganisms and the contaminants, faster degradation rate more than other biological treatments, provides greater control of environmental and operating conditions, and gas emissions are controlled and harnessed as biogas and requires small site space. The disadvantages include; it is an *ex situ* process and requires soil excavation, dewatering of soil after treatment is required and can be expensive, the treatment cost is high when off-gas is treated due to volatile compounds, and sizing materials is difficult and expensive as non-homogeneous soil and clayey soil create materials handling issues, and further treatment of non-recycled effluent is required [82].

2.9 Landfarming

Landfarming, also known as land treatment or land application, is an above-ground form of bioremediation technology that involves engineered bioremediation systems that employ tilling, ploughing, and spreading the polluted soil in a thin layer on the land surface to enhance and stimulate aerobic microbial activities with the addition of nutrients, mineral and moisture to reduce the pollutant level.
biologically [86]. It is suitable for treating soil contaminated with low molecular weight petroleum hydrocarbons, volatile organic compounds (VOCs), and other organic compounds [88]. Enhancing biodegradation in landfarming is achieved by adding oxygen, moisture and nutrients [89]. Tilling also introduces oxygen to the soil and helps increase evaporation while adding nutrients or soil amendments such as organic wastes or organic fertilisers provide nutrients to stimulate microbial activities [90]. Figure 10 illustrates the component in the landfarming system for petroleum hydrocarbon contaminated soil.

The Landfarming method has been proven effective in reducing all the constituents of petroleum hydrocarbons at underground storage tanks. Low molecular hydrocarbons tend to be removed by volatilisation during landfarming aeration, tilling and ploughing and degraded through microbial respiration. The heavy molecular hydrocarbons do not volatilise during landfarming aeration but undergo breakdown by biodegradation activity by the soil microorganism [66].

The study demonstrated by Brown et al. [88] showed landfarming to improve biological treatment of petroleum hydrocarbons in the soil in 110 days with nutrient addition. The results obtained after 6 weeks showed 53% for total petroleum hydrocarbon (TPH) removal from the contaminated soil. Landfarming is a successful treatment option for remediation of petroleum hydrocarbon contaminated soil.

The benefits of landfarming treatment include; low capital input, simple technology design and implementation, a large volume of polluted soil can be treated, in situ and ex situ application, negligible environmental impact and energy efficiency. The disadvantages include; it is limited to removal of biodegradable pollutants, a large treatment area is required, involves pollutant exposure risks, excavation incurs additional cost, and it provides limited knowledge of the microbial process or the unravelling limitation factors during remediation [91].

2.10 Bio-piling

Bio-piles, also known as bio-cells, bio-heaps, bio-mounds and compost piles, are used to reduce the concentrations of hazardous petroleum hydrocarbon contaminants
in excavated soils through biodegradation. The technology involves a combination of landfarming and composting in an engineered cell aerated with blowers and vacuum pumps, irrigation and nutrient system, and leachate collection system for bioremediation of pollutant components adsorbed to soil and sediments [92]. The technique involves piling an excavated contaminated soil, followed by biostimulation and aeration to enhance microbial activities for degradation [93]. It is suitable for treating a large volume of contaminated soil and sediments in a limited space and effectively remedy pollutions in extreme environments [94, 95].

The essential components of the technique include the addition of air (oxygen), moisture (water), nutrients and bulking agents (organic materials), leachate collection system and treatment bed [96]. Biopiling of contaminated soil can limit the volatilisation of low molecular weight contaminants in petroleum hydrocarbons [97]. Biopile systems are similar to landfarms in that they are both engineered and above-ground systems that use oxygen from the air to stimulate the growth and reproduction of aerobic microorganisms, which degrade the adsorbed petroleum hydrocarbon contaminants in the soil. While landfarms are aerated through tilling or ploughing, biopiles are aerated through air injection or extraction through slotted or perforated piping placed throughout the piles [66]. Figure 11 illustrates the biopiling process for remediation of petroleum hydrocarbon contaminated soil.

Gomez and Sartaj [98] demonstrated a study by conducting biopiling treatment of petroleum hydrocarbon contaminated soil at a low-temperature field scale using consortia of microorganisms and organic compost for 94 days. The result obtained showed a removal efficiency of 90.7% for total petroleum hydrocarbon (TPH).

The benefits of biopiling include; it is relatively simple to design and implement, effective for pollutants with slow biodegradation rates, it can be designed to be a closed system with vapour emission controls, it requires less land area than landfarms, and cost-effective. The limitations include; contaminants reduction >95% and concentration <0.1 ppm are challenging to achieve, not practical for high pollutant concentrations, volatile compounds tend to evaporate rather than biodegrade during treatment, a large land area is required, vapour generation require treatment before discharge, and requires bottom liners to prevent leaching [66].

2.11 Composting

Composting is a controlled microbial aerobic biochemical degradation of organic waste materials and its conversion into a stabilised organic material that can be useful as soil conditioners for remediation of soil contaminated with organic compounds such as petroleum hydrocarbons [99, 100]. The composting process involves careful control with nutrient addition, tilling, watering and addition of suitable microbial consortia and bulking materials in the form of organic wastes to improve bioremediation. The composting process requires thermophilic conditions of 50–65°C to properly compost soil contaminated with hazardous compounds such as petroleum hydrocarbon compounds. An increased temperature results from heat generated from the microbial activities during the metabolic breakdown of organic materials in the compost, and efficient degradation of pollutants is achieved by periodic tilling, watering and aeration of the compost [101]. Figure 12 illustrates the compost piling of contaminated soil.

Atagana [102] conducted composting bioremediation of petroleum hydrocarbons using sewage sludge compost on contaminated soil with a total petroleum hydrocarbon (TPH) concentration of 380,000 mg kg\(^{-1}\) for 19 months. The results obtained
after the experiment period showed a 99% removal efficiency for TPH, while other selected hydrocarbon components were removed 100% within the experiment period. Composting helps degrade, bind and convert contaminants into harmless substances and compounds with substantial potential for remediation application to treat petroleum hydrocarbon contaminated soil [103].

The benefits of compost piling include abundant nutrients, soil enrichment retains moisture and nutrients, improves soil quality and altering soil pH, cheap soil conditioner, eco-friendly and cost-effective, and promoting the growth of beneficiary microorganisms. The disadvantages include; it requires extensive monitoring and turning of the pile, takes time and energy, takes about 6 months to
2 years under optimal conditions, emission of greenhouse gases and requirement for a large site area.

2.12 Windrow

The windrow treatment process relies on periodic tilling, ploughing and turning piled contaminated soil with water application to increase moisture and aeration with the distribution of nutrients to enhance biodegradation. In the windrowing process, the increase in microbial activities by the indigenous and transient petroleum hydrocarbon-degrading microorganisms in the contaminated soil speed up the biodegradation process [71, 79]. The biodegradation process is accomplished through biotransformation, assimilation and mineralisation [104]. Compared with biopiling, the windrowing method showed a higher removal efficiency rate for petroleum hydrocarbons. The windrowing process for the remediation of polluted soil is illustrated in Figure 13.

A study demonstrated by Al-Daher and Al-Awadhi [105] investigated biodegradation of petroleum hydrocarbon contaminated soil using a windrow soil system for 10 months. The windrow system was subjected to regular watering, tilling and turning to enhance aeration and microbial activities. The results obtained showed a 60% reduction in the total petroleum hydrocarbons (TPH) in the first 8 months, and the degradation rate was enhanced when the moisture content was effectively maintained.

The benefits of the windrowing process include; soil enrichment, retaining moisture and nutrients, improving soil quality and altering soil pH, requiring low capital and operational costs, being eco-friendly and easy to implement and promoting the growth of beneficiary microorganisms. On the downside, windrow treatment is not the best option in removing soil contaminated with volatile petroleum hydrocarbon compounds due to the release of toxic volatile compounds during the periodic turning and tilling [79]. There is an emission of greenhouse gases such as methane (CH4) in windrow treatment due to the formation of an anaerobic zone within the piled heap [103]. It requires ample space for composting, attracting scavengers, long duration of time under optimal conditions, produces odour, compost may become anaerobic in rainy conditions, requires regular turning to maintain aerobic conditions and vulnerability to climate changes.

![Figure 13](image)

**Figure 13.**
Windrowing of petroleum hydrocarbon polluted soil.
2.13 Vermiremediation

Vermiremediation is an expanding technology that uses earthworms to biodegrade hazardous contaminated soil [106, 107]. The earthworms in the soil help enhance and improve soil fertility, biological, chemical and physical properties. They stimulate and enhance microbial activities by creating suitable conditions for microorganisms to thrive and improve soil aeration by burrowing and tunnelling through the soil structures [108, 109]. The presence of earthworms in the soil depends on soil moisture, organic matter content and pH. They usually occur in diverse habitats, especially those rich in organic matter and moisture [110, 111]. Vermiremediation of petroleum hydrocarbon in the soil occurs through vermidegradation. The earthworms stimulate the biodegradation processes by enhancing oxidation, soil aeration and microbial activities in the polluted soil. Figure 14 illustrates the components of vermiremediation in petroleum hydrocarbon contaminated soil.

A study demonstrated by Azizi et al. [113] conducted vermiremediation using earthworm (Lumbricus rubellus) to degrade petroleum hydrocarbon components such as polycyclic aromatic hydrocarbons (PAHs), anthracene, phenanthrene and benzo[a]pyrene (BaP) within 30 days. The result obtained showed a removal efficiency of 99.9% for PAHs. Sinha et al. [114] demonstrated a similar study for earthworms remedial action on polycyclic aromatic hydrocarbons (PAHs) contaminated soils in a gasworks site. The result obtained showed 80% removal efficiency for PAHs compared to 21% removal efficiency in microbial degradation.

The benefits of vermiremediation include; minimal environmental disruption, enhanced organic matter, nutrient concentration and biological activity, improved soil utility and fertility, and cost-efficiency. The disadvantages include; high concentration of pollutants may be toxic to the earthworms, the process is restricted to the depth of earthworm activities, effective for slightly or moderately contaminated soil,
requires strict conditions, sensitive to climate and seasonal conditions, and restricted by food abundance in the soil [106].

2.14 Mycoremediation

Mycoremediation involves using fungi processes to biodegrade hazardous contaminants such as petroleum hydrocarbons to less toxic or non-toxic forms, thereby reducing or eliminating environmental contaminants [115–117]. Fungi can degrade variable environmental recalcitrant pollutants due to their ability to produce and secrete extracellular enzymes such as peroxidases that break down lignin and cellulose [118, 119]. Ligninolytic fungi such as the white-rot fungi *Polyporus* sp. and *Phanerochaete chrysosporium* are essential in mycoremediation because they can degrade a diverse range of toxic and hazardous pollutants [120]. The degradative action of fungi is effective in various situations where they degrade different materials. When cultivated in polyethylene contaminated soil, fungi such as *Penicillium* sp. degrade polyethylene effectively. [121]. Figure 15 illustrates the mycoremediation components in petroleum hydrocarbon polluted soil.

Studies have shown that many filamentous fungi species are petroleum hydrocarbon-degrading in nature. Some white rot fungi use their mycelia to degrade petroleum hydrocarbon contaminants due to their high production of oxidative enzymes, extracellular enzymes, chelators and organic acids, which help them degrade petroleum hydrocarbon pollutants [122]. In a mycoremediation study demonstrated by Ulfig et al. [123], keratinolytic fungi *Trichophyton ajelloi* were utilised to remove hexadecane and pristane from crude oil-polluted soil. In another similar study conducted by Njoku et al. [107], *Pleurotus pulmonarius* was used in mycoremediation of soil contaminated with petroleum hydrocarbon mixture comprising petrol, diesel, spent engine oil and spent diesel engine oil lubricant at the ratio of 1:1:1:1 in various concentrations of 2.5%, 5%, 10% and 20% for 62 days period. The results showed that the soil with 10% concentration had a removal efficiency of 68.34% for TPH, while soil with 2.5% concentration yielded 22.12% removal efficiency for TPH. These
results suggest that the fungi *Pleurotus pulmonarius* can biodegrade soil contaminated with a moderate level of the petroleum hydrocarbon mixture.

The benefits of mycoremediation include; minimal disturbance to the environment, does not produce corrosive or harmful chemicals, eco-friendly and cost-effective, and requires no special equipment. The disadvantages include; the efficiency is not 100%, long-duration for treatment, periodic turning with reapplication of growth medium is required, competition with indigenous bacterial population may reduce the efficiency, and high concentration of contaminants may be toxic to the fungi.

### 2.15 Phycoremediation

Phycoremediation, a technique that uses algal species (macroalgae or microalgae) to sequester, remove, break down, biotransform or metabolise pollutants such as petroleum hydrocarbons from contaminated water environments [124–126]. As illustrated in Figure 16, this technique is one of the effective methods used in water pollution treatment due to its high efficiency and low-cost usage [127]. Algae can accumulate and degrade toxic pollutants and organic compounds such as petroleum hydrocarbons, biphenyls, pesticides, and phenolics [125]. Algae are very adaptive in most environments and grow in autotrophic, mixotrophic, or heterotrophic conditions. Algae play a vital role in regulating and controlling the concentration of metals in the water environment. The mixotrophic algae are excellent in bioremediation and carbon sequestration [128].

Algae can produce O$_2$, fix CO$_2$ by photosynthetic process, increase the BOD level in the polluted water, and remove excess nutrients [129]. The mineral uptake by microalgae occurs in two steps. The initial step is independent of cell processes and involves physical adsorption onto the cell's surface, and the ions are gradually carried into the cell by chemisorption [120]. The second step is dependent on cell processes and involves intracellular uptake and absorption. Studies have shown that heavy metals can be sequestered in the polyphosphate body of algae and serve for detoxification and storage [130]. Phycoremediation was successfully used to reduce nutrient levels in wastewater treatment, and the technique includes algal biofilm, algal turf scrubbers,

![Figure 16. Phycoremediation technique in a pond system.](image-url)
high-rate algal ponds, and immobilised algae [127]. Several algae species such as *Chlamydomonas*, *Chlorella*, *Botryococcus* and *Phormidium* are involved in phycoremediation. The use of microalgae in the phycoremediation of petroleum hydrocarbon is gaining interest as some algae species can degrade and oxidise hazardous petroleum hydrocarbon components into less noxious compounds [131, 132].

A phycoremediation study was demonstrated by Kalhor et al. [133], who investigated the potential of *Chlorella vulgaris* in biodegradation of the crude oil-contaminated water environment. Different crude oil concentrations were prepared and treated in their investigation, and the removal efficiency was calculated after the incubation period. The result obtained after 14 days incubation period showed that aromatic hydrocarbon compounds (benzene and naphthalene) and alkane (nonadecane) were biodegraded at the removal efficiencies of 89.17% at 10 g/l and 76.53% at 20 g/l concentration by the algae. Their result confirmed that the algae *C. vulgaris* could remove light components of petroleum hydrocarbon compounds in the contaminated water.

The advantages of phycoremediation include; simple and economic pilot scale, low implementation cost, high versatility and adaptability, high nutrient removal in effluents, algal biomass is easy and cheap to harvest in low scale operation, and the algal biomass can be used for biogas production. The disadvantages include; it is difficult and expensive to harvest algal biomass in large scale operations, poor and inconsistent contaminant removal due to characteristics of the pollutants, sensitivity to climate and seasonal conditions, the infestation of predators that feed on algae, and injection of CO$_2$ incur a cost for the implementation.

### 2.16 Phytoremediation

Phytoremediation is a low-cost remediation technique that uses green plants and the associated soil microorganisms to reduce the concentrations of contaminants and their toxic effects [134]. The technique removes, extracts, and sequesters the contaminants (decontamination) into the plant matrix (stabilisation) [43]. Phytoremediation uses the natural processes of the green plants or plant-based systems to remediate environments contaminated by organic compounds, heavy metals, and inorganic compounds. It formed the basis of the reed beds and constructed wetlands [43]. The phytoremediation system uses the synergistic relationship among the plants, indigenous microorganisms dwelling in the contaminated soil, and the roots of the plants [135]. The plants produce inherent enzymatic activities and uptake processes that remove and sequester contaminants. The plants act as symbiotic hosts to aerobic and anaerobic microorganisms, providing nutrients and habitat to the microorganisms [134]. The mechanisms of phytoremediation include phytoextraction (phytoaccumulation), phytodegradation, phytostabilisation, phytotransformation, phytovolatilisation, rhizofiltration, and rhizodegradation (rhizoremediation), as illustrated in Figure 17 [137, 138].

In phytoremediation, plants break down, degrade, concentrate, sequester, bioaccumulate, contain, stabilise and metabolise contaminants by acting as filters or traps in the tissue through various mechanisms. These mechanisms convert the contaminants into less toxic and less persistent in the environments [139]. The mechanisms and efficiency of the phytoremediation technique depend on the pollutants, bioavailability, and properties of the polluted soil, and the mechanisms affect the mobility, toxicity of pollutants, volume, and concentration [136, 140]. The plants’ roots and shoots provide colonisable surface area for absorption, exudates, and leachates in the rhizosphere for microbial activities [141]. The success of phytoremediation depends
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mainly on the plant’s ability to bioassimilate or bioaccumulate both organic and inorganic contaminants into their cell wall structures and carry out oxidative degradation of organic xenobiotics [142].

Many researchers have conducted phytoremediation and reported studies using different plants to remediate soil contaminated with petroleum hydrocarbons, heavy metals and other organic pollutants. Cook and Hesterberg [143] published a summary of major plants (trees and grasses) currently used in phytoremediation, which adsorb or degrade contaminants in polluted environments. Other researchers, including Dadrasnia and Agamuthu [144], Cartmill et al. [145] and Agamuthu et al. [146], demonstrated phytoremediation of petroleum hydrocarbon contaminated soil using several plants with the addition of organic wastes and organic fertilisers to enhance the biodegradation process.

Some of the advantages of phytoremediation include; it is a permanent treatment technique, it has low capital investment and operation costs, there is no soil excavation, phyto-accumulated metals may be recycled and provides additional economic advantages, it eliminates secondary air and water-borne wastes, and it has public acceptance due to aesthetic reasons. The disadvantages include being slower than other remediation techniques, hyperaccumulating plants being slow growers, working efficiency is not 100%, may not be effective for mixture pollutants, high concentration of contaminants may be toxic to plants, and treatment is limited to shallow contaminants.

2.17 Electrobioremediation

Electrobioremediation or bioelectrochemical system is an emerging biodegradation technology with a trans-disciplinary system that depends on the use of
electroactive microorganisms to catalyse the oxidation or reduction reactions of organic and inorganic electron donors. The bioelectrochemical system delivers electrons to the solid-state electrode (anode), with subsequent transfer or exchange of electrons to the solid-state electrode (cathode) through a conductive circuit and simultaneously generating electrical energy (Figure 18) [147, 148]. The mechanism involves an electrokinetic process in the acceleration and orientation of the transport of pollutants and microorganisms [149].

Bioelectrochemical system works effectively in contaminated media as unlimited electron acceptors or donors [150] and converts chemical energy from organic wastes or contaminants to electrical energy and hydrogen or value-added chemical products [151]. The system works on the interface of electrochemistry and fermentation [152]. The bioelectrochemical system can be classified based upon the application of microbial fuel cells for power generation, microbial electrolytic cells for biofuel production, microbial desalination cell for saline water desalination, and microbial electro synthetic cells for the synthesis of value-added by-products [134].

A study conducted by Daghio et al. [77] demonstrated that bioelectrochemical systems energised and stimulated anaerobic oxidation of different types of organic wastes to reduce contaminants in soil and groundwater, including petroleum hydrocarbons halogenated compounds. In a laboratory study, Palma et al. [153] demonstrated a bioelectrochemical treatment system for petroleum hydrocarbon contaminated groundwater. The results showed that phenols were gradually removed from 12 to 50% while electric current generation gradually increased from 0.3 mA to 1.9 mA. The phenol removal rate and the coulombic efficiencies were $23 \pm 1$ mg L$^{-1}$ d and $72 \pm 8\%$ on average.

The advantages of electrobioremediation include generating electrical energy level and electron flux; no waste is generated, cheap operational cost, and highly selective

![Figure 18. In situ electrobioremediation of oil-polluted soil [77].](image-url)
towards target pollutants, pollutants can be adsorbed on the electrodes when graphite or carbon is used. The disadvantages include; slower anaerobic degradation than aerobic degradation. The cathodic reaction may limit the anodic reaction when microbial fuel cells are used, chlorine gas is produced, a scale-up process is challenging, and the process is affected by changes in pH in the contaminated soil [77].

2.18 Nanobioremediation

Nanobioremediation is an emerging technology used in remediating environmental pollutions. The system functions with the aid of reactive biosynthetic nanomaterials (NMs), nanoparticles (NPs), nanostructured materials (NSMs), nanocomposites manufactured particles (NCMPs), manufactured nanoparticles (MNPs), and nanoclusters (NCs) [154–156]. These biosynthetic nanoparticles exhibit unique physical, chemical and biochemical properties in enzyme-mediated remediation, transformation, and detoxification of persistent hydrophobic contaminants and toxicants [157]. These nanomaterials or particles are engineered or formed by plants or microorganisms and comprise particles with at least one dimension measuring between 1.0 and 100 nm [158, 159]. Figure 19 illustrates in situ nanobioremediation of oil-polluted soil.

The nanoparticles can be carbon-based (carbon fullerenes) and carbon nanotubes. They can be metal-based (quantum dots, nano zero-valent iron (nZVI), nanosilver, nanogold, and nanosized metal oxides such as ZnO, Fe$_3$O$_4$, TiO$_2$, CeO$_2$). They can also be dendrimers or nano polymers and composite or bulk-type materials [161]. The nanomaterial or nanoparticles have properties that allow catalysis and chemical reduction to remove the contaminants. As reducing agents, the particles degrade hazardous organic contaminants in the environment. The process changes elements’ oxidation state, combined with catalytic enhancement of redox reactions for soil and groundwater remediation.

In the nanoremediation process, no groundwater is pumped out for above-ground treatment, and no soil is excavated or transported to a different location for disposal.
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and treatment [162]. With the nanoparticles’ minute size and innovative surface coating, they pervade tiny spaces in the subsurface and remain dispersed in the soil or groundwater, allowing the particles to move and migrate farther than larger or micro or macro-sized particles and achieve wider distribution [163]. The sorption process occurs by adsorption and absorption. In adsorption, the interactions between the pollutants and the sorbent occur at the surface level, while in absorption, the pollutants penetrate deeper into the sorbent layers to form a solution [164]. The mobility of natural or biosynthetic nanoparticles depends on their dispersions, aggregations, settlings, and formation of mobile clusters.

Nanoparticles such as zeolites, carbon nanotubes, nanofibres, metal oxides, titanium dioxide, enzymes, and noble metals such as bimetallic nanoparticles (BNPs) have been used successfully in the remediation of organic compounds and petroleum hydrocarbons from the contaminated environments [165, 166]. Among the nanoparticles, the most widely used is the nanoscale zero-valent iron (nZVI) modified with palladium inclusion as a catalyst for improved performance [167]. Nanobioremediation can be used where other conventional remediation technologies do not prove productive because nanoparticles are less toxic to soil flora and enhance microbial activity [157]. The nanoparticles have highly desired properties for in situ applications due to the nanosize and innovative surface coatings. The particles easily penetrate tiny spaces in the subsurface, remain suspended in groundwater, and allow further migration and wider distribution [163].

A study conducted by Reddy et al. [168] demonstrated nanobioremediation using nanoscale iron to degrade the organic compound dinitrotoluene (DNT) in the soil. The results obtained showed 41–65% removal efficiency for DNT near the anode, while removal efficiency of 30–34% was recorded near the cathode. The highest removal was recorded using lactate-modified nanoscale iron particles. However, the overall degradation of DNT was due to nanoscale iron particles having the electrochemical process that enhanced the delivery of nanoscale particles in the degradation of organic contaminants.

The advantages of nanobioremediation include; effectivity across a wide range of environmental conditions, the high surface area increasing reactivity and treatability, extending the range of treatable contaminants, eliminating intermediate by-products, and combining with other treatment techniques for enhanced remediation. The disadvantages include; potential to generate harmful by-products, the potential to enter the food chain with the possibility of biomagnification and bioaccumulation, the production of nanoparticles is an expensive engineering process, and the societal issue due to fear of the environmental impact from the manufactured nanoparticles.

2.19 Trichoremediation

Trichoremediation is an emerging technique. The etymology originates from the ancient Greek word θρίξ (tricho), meaning “hair,” and Latin word (remedium), meaning “restoring balance.” It describes a biological treatment of environmental contaminants by utilising hairs (keratinaceous materials) to increase the metabolic activities of the keratinolytic and keratinophilic microbes with pollutant degrading abilities in the co-metabolic degradation of the substrates [134]. The microorganisms display lipolytic activity and remove petroleum hydrocarbons from the medium during biodegradation [123, 169]. Trichoremediation involves biostimulation of indigenous microorganisms in the contaminated soil and bioaugmentation with the naturally associated microorganisms inhabiting the hair materials. Additional mechanisms that
participate in the process are absorption and adsorption due to the chemisorption properties of hairs [170–172]. Figure 20 illustrates the components of trichoremediation for petroleum hydrocarbon contaminated soil.

Cervantes-González et al. [173] investigated the ability of chicken feather wastes as petroleum hydrocarbon sorbent and studied their structural biodegradation and removal of petroleum hydrocarbons. Their findings showed that chicken feathers enhanced the contact between petroleum hydrocarbons and bacteria and enhanced the removal of petroleum hydrocarbons. They also observed that the microorganisms colonised the chicken feathers and degraded the materials completed in the study. In their observation during the treatment, there was an exponential growth phase of bacteria during the early days of the treatment, and the simultaneous degradation of feathers and petroleum hydrocarbons was evident [173].

The benefits of trichoremediation technology include; relatively low cost and maintenance, ease of implementation and operation, reduced landfill wastes, fully organic and biodegradable materials, improved soil quality and structure, and additional accessible carbon sources and co-metabolites. The disadvantages include; long treatment time, sensitivity to the level of toxicity and environmental conditions, generating toxic metabolites, metabolic pathways may switch to a less toxic carbon source, inhibits metabolic pathway by the presence of the metabolites, and additional compounds may negatively affect the biodegradation process.

3. Factors affecting the biological treatment technologies

The purpose of biological treatment technologies for biodegradation of petroleum hydrocarbon polluted sites through sustainable and eco-friendly means is to eliminate the hazards of pollution in the environment and human health risks. Applying biological treatment technology in a polluted environment at a field scale is a challenging and laborious task. The choice of a biological treatment technology
depends on several biological and environmental properties, which vary from one site to another. The influencing parameters comprised environmental and biological properties include nature and concentration of the contaminants, type and properties of the soil, and the interaction with microorganisms and metabolic pathways. [174]. The environmental properties influence the biological properties, while the biological properties produce the overall biodegradation effect in the system. The environmental properties affecting biodegradation influence the rates and extent of microbial transformation of the pollutants [175]. Biological treatment technologies immobilise contaminants through adsorption, absorption, desorption, volatilisation, solubilisation, complexation, hydrolysis, oxidation, and mineralisation [12, 13]. Figure 21 illustrates the various factors affecting biological treatment technologies.

4. Conclusions

The biological treatment technologies have grown as alternatives to the traditional physicochemical, thermal and electromagnetic technologies for the remediation
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of petroleum hydrocarbons polluted soil. They are preferred due to low energy consumption, cost-effectiveness, environmental-friendliness, non-invasiveness, feasibility, and sustainability compared to other physicochemical, thermal and electromagnetic treatment options, which are cost-prohibitive, often destroy the soil properties and render the soil impoverished and sterile eventually. The biological treatment technologies can be selectively adapted and adopted to degrade the pollutants without causing further damage to the site and the indigenous flora and fauna. Although various biological treatment technologies are accessible, no single biological treatment is the most suitable for all varieties of contaminants and the type of site-specific conditions occurring in the petroleum hydrocarbon-affected environments. Good knowledge of the environmental conditions of the affected environments, nature, composition and properties of the contaminants, fate, transport, and distribution of the contaminants, mechanism of biodegradation, the interactions and relationships with the microorganisms, intrinsic and extrinsic factors affecting the remediation processes, and the potential impact of the possible remedial measure determine the choice and selection of a biological treatment technology requirements. More than one biological treatment technology may be adopted or combined into a process train to effectively remove, contain or destroy the petroleum hydrocarbon pollutants in polluted environments.

However, selecting one or more biological treatment technology is essential in decision-making, as many parameters that conflict in nature plays a significant role in decision-making. Consequently, it is a welcome idea to select biological treatment technologies that are feasible, adaptive, scientifically defensible, sustainable, non-invasive, eco-friendly, and economical because remediation of petroleum hydrocarbon polluted environments through the conventional physicochemical, thermal, and electromagnetic technologies is a challenging, laborious, extensive and expensive task.

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**Conflict of interest**

The authors hereby declare that there is no conflict of interest regarding the publication of this book chapter.
Author details

Innocent Chukwunonso Ossai$^{1,2*}$, Fauziah Shahul Hamid$^{1,2}$ and Auwalu Hassan$^{1,2,3}$

1 Faculty of Science, Institute of Biological Sciences, University of Malaya, Kuala Lumpur, Malaysia

2 Faculty of Science, Centre for Research in Waste Management, University of Malaya, Kuala Lumpur, Malaysia

3 Faculty of Science, Department of Biological Sciences, Federal University Kashere, Gombe State, Nigeria

*Address all correspondence to: ossainonso@yahoo.com

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