Application of Biological Methods in the Remediation of Oil Polluted Environment in Nigeria

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Review Article

ABSTRACT

The application of biological methods have been used to remediate oil contaminated land, to promote health and safety of our environments. These biological processes of remediation may be affected by use of single or a combination of approaches. These processes include to degrade, break down, transform, and/or essentially remove contaminants or impairments of quality from soil and water, otherwise known as bioremediation. Bioremediation is a natural process which relies on bacteria, fungi, and plants to alter contaminants as these organisms carry out their normal life functions. Metabolic processes of these organisms are capable of using chemical contaminants as an energy source, rendering the contaminants harmless or less toxic products in most cases. This paper is a review of the biological methods used in the remediation process.

1. INTRODUCTION

Frequent oil spill incidents have become a problem to ecological protection efforts. Over 6,000 oil spills had been recorded in the 40 years of oil exploitation in Nigeria giving an average of 150 spills per annum. A total of 4,647 incidents of oil spills occurred between 1976 and 1996 resulting in the spillage of about 2,369,470 barrels of crude oil and only about 549,060

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barrels were recovered, 1,820,410 barrels were lost to the ecosystem [1]. Between 2006 and 2012 alone a total of 127,467.96 barrels of oil were spilled [2]. The oil reduces the soil's fertility such that most of the essential nutrients are no longer available for plant and crop utilization [3]. The concomitant effects of this pollution on the physical, chemical and biological components of the environment are many and adverse in form. Oil spillage generally contaminates the soil and water, affect their productivity, and distort the aesthetic value of the ecosystems [4]. The soils which harbour microorganisms as well as plant roots serve as one of the dynamic sites of biological interactions in nature. Many of the biochemical reactions that enhance mineralization of soil organic matter and nutrition of plants that are mediated by organisms are affected by the degradation of soil environment [4,5]. In this respect microbial activities are adversely effected and so, plant growth and detoxification of pollutants are also impaired. The damage to the ecosystems impacts adversely on the socio-economy and livelihood of the people [6,7]. With increasing demand for food orchestrated by a rising population, the spate of pollution of arable lands is an unacceptable risk to agricultural production, ecosystems, human health, good practices as well as sustainable development. The use of synthetic remediation formulations in the amendment of oil spill sites are expensive and could have attendant negative environmental effects on the resident biota. Bioremediation functions basically on biodegradation, which involves complete mineralization of organic contaminants into carbon dioxide, water, inorganic compounds, and cell protein or transformation of complex organic contaminants to other simpler organic compounds by biological agents like microorganisms. This paper therefore focuses on the application of biological methods to remediate oil contaminated environments by the action of organic compounds and microorganisms.

2. BIOLOGICAL METHODS

2.1 Bioremediation

Many substances known to have toxic properties are regularly introduced into the environment through human activity. These substances range in degree of toxicity and danger to human health. Many of these substances either immediately or ultimately come in contact with or are sequestered by soil. Conventional methods to remove, reduce, or mitigate toxic substances introduced into soil or ground water via anthropogenic activities and processes include pump and treat systems, soil vapor extraction, incineration, and containment. Utility of each of these conventional methods of treatment of contaminated soil and/or water suffers from recognizable drawbacks and may involve some level of risk.

Bioremediation offers an alternative method to detoxify contaminants and is being used as an effective means of mitigating hydrocarbons, halogenated organic solvents and compounds, non-chlorinated pesticides and herbicides, nitrogen compounds, metals (lead, mercury, chromium) and radionuclides.

Bioremediation technology exploits various naturally occurring processes which include; natural attenuation, biostimulation, and bioaugmentation. Natural attenuation occurs without human intervention other than monitoring but rather relies on natural conditions and behavior of soil microorganisms that are indigenous to soil. Biostimulation also utilizes these indigenous microbial populations to remediate contaminated soils and consists of adding nutrients and other substances to soil to catalyze natural attenuation processes. Bioaugmentation involves introduction of exogenic microorganisms (sourced from outside the soil environment) capable of detoxifying a particular contaminant, sometimes employing genetically altered microorganisms [8,9].

During bioremediation, microbes utilize chemical contaminants in the soil as an energy source and, through oxidation-reduction reactions, metabolize the target contaminant into useable energy for microbes. The by-products (metabolites) released back into the environment are typically in a less toxic form than the parent contaminants. For example, petroleum hydrocarbons can be degraded by microorganisms in the presence of oxygen through aerobic respiration. The hydrocarbon loses electrons and is oxidized while oxygen gains electrons and is reduced. The result is formation of carbon dioxide and water [10]. When oxygen is limited in supply or absent, as in saturated or anaerobic soils or lake sediment, anaerobic (without oxygen) respiration prevails. Generally, inorganic compounds such as nitrate, sulfate, ferric iron, manganese, or carbon dioxide serve as terminal electron acceptors to facilitate biodegradation [11,12].

Three primary ingredients for bioremediation are: 1) presence of a contaminant, 2) an electron acceptor, and 3) presence of microorganisms that are capable of degrading the specific contaminant. Generally, a contaminant is more easily and quickly degraded if it is a naturally occurring compound in the environment, or chemically similar to a naturally occurring compound, because microorganisms capable of its biodegradation are more likely to have evolved [13]. Petroleum hydrocarbons are naturally occurring chemicals; therefore, microorganisms which are capable of attenuating or degrading hydrocarbons exist in the environment.

In situ bioremediation causes minimal disturbance to the environment at the contamination site. In addition, it incurs less cost than conventional soil remediation or removal and replacement treatments because there is no transport of contaminated materials for off-site treatment. However, *in situ* bioremediation has some limitations: 1) it is not suitable for all soils, 2) complete degradation is difficult to achieve, and 3) natural conditions (i.e. temperature) are hard to control for optimal biodegradation. *In-situ* bioremediation for water logged soils can be treated at a target concentration of 200 mg kg^{-1} in about 1-2 years of continual operation at ambient temperatures [14].

Ex situ bioremediation approaches include use of bioreactors, land farming, and biopiles. In the use of a bioreactor, contaminated soil is mixed with water and nutrients and the mixture is agitated by a mechanical bioreactor to stimulate action of microorganisms. This method is better-suited to clay soils than other methods and is generally a quick process [15].

2.2 Natural Attenuation

Natural attenuation processes refers to reliance on natural processes to achieve site –specific remedial objectives. This involves removal of the source of contamination as far as practicable. The processes include a variety of physical, chemical or biological processes which under favourable conditions, can reduce the mass toxicity, mobility, volume or concentration of contaminants in soil or water resources. These include biodegradation, dispersion, dilution, sorption, volatilization and chemical or biological stabilization, transformation or destruction of contaminants [15]. The preliminary stages in the natural attenuation process include; excavation of soil, tiling of soil, site preparation before nutrient enrichment for recovery (i.e. biostimulation) using fertilizers.

Excavation of the soil to at least one meter depth, thus creating a treatment cell so that run-offs can be collected by scooping. Secondly, tiling is necessary to ensure acceleration of natural removal of crude oil thus exposing oil stained soils to oxygen, thus providing the essential contact between oil degrading bacteria and contaminated soils. Site preparation involves turning over or ploughing of impacted soils to a depth of 0.3m, homogenization of broken loosely compacted sandy and clay soil lumps to a depth of 0.3m before nutrient enrichment using appropriate levels of organic fertilizer application.

Natural attenuation is usually limited by low nutrients, aeration, low temperatures and water availability. For aeration, treatment can be facilitated in an air sparge port to increase oxygen concentration in the soils, because the shallow water table and thin soil cover will help channel development [16] or micro- venting system comprising many small air injecting rods may be installed to aerate a wide range area of soil. Nitrogen requirements are needed to maximize petroleum bioremediation in soils.

2.3 Land Farming

This is a bioremediation treatment process that is performed in the soil zone or in bio-treatment cells, whereby soils, sediments, or sludges are incorporated into the soil surface and periodically turned over (tilled) to aerate the mixture [12], [16]. This technique has been successfully used for years in the management and disposal of oily sludge and other petroleum refinery wastes. *In situ* systems have been used to treat near surface soil contamination for hydrocarbons and pesticides [17]. The equipment employed in land farming is typical of that used in agricultural operations.

Land farming involves spreading contaminated soil over a collection system and stimulating microbial activity by allowing good aeration and by monitoring nutrient availability [14]. Use of land farming and biopiles also present the issue of monitoring and containing volatilization of contaminants. Conventionally, land farming method could be expected to bring about amendment of contaminated sites in a shorter duration of time when combined with the introduction of cheap and locally sourced organic manures.

2.4 Bioreactor (Biochemical Engineering)

A bioreactor refers to any device or system that supports a biologically active environment. It could be a vessel in which a chemical process that involves microorganisms or biochemically active substances derived from such organisms, which metabolize into products and cause modification of the interfacial properties of the bioreactor system with the aim of facilitating oil movement through porous `media. In such a system, the microbial activity will affect fluidity (viscous reduction and miscible reduction); displacement efficiency (decrease of interfacial tension, increase in permeability); sweep efficiency (mobility control and selective plugging) and drive force (reservoir pressure) [18]. This process can either be aerobic or anaerobic. These bioreactors are commonly cylindrical, ranging in size from litres to cubic meters, and are often made of stainless steel [19]. A bioreactor may also grow cells or tissues in the context of cell culture. These devices are being developed for use in tissue engineering. Organisms growing in bioreactors may be suspended or immobilized [19]. In a petri dish with agar gel. Bioreactors could be used in sewage treatment and other biochemical engineering application [20]

2.5 Composting

Composting is the decaying of food, mostly vegetables or manure. It involves the aerobic decomposition of biodegradable organic matter, and is performed primarily by facultative and obligate aerobic bacteria, yeast and fungi. Composting recycles or "down cycles" organic household and yard waste such as fruits, vegetables and manures into an extremely useful humus-like, soil end-product called compost. [18]. Ultimately this permits the return of needed organic matter and nutrients into the food chain and reduces the amount of "green" waste going into landfills [21]. Composting is widely believed to considerably speed up the natural process of decomposition as a result of the higher temperatures generated [22]. The elevated heat results from exothermic processes, and the heat in turn reduces the generational time of microorganisms, and thereby speeds up the energy and nutrient exchanges taking place.

2.6 Biodegradation

Biodegradation is a mechanism by which petroleum and other hydrocarbon pollutants can be removed from the environment by natural populations of microorganisms [23]. One important requirement is the presence of microorganisms with the appropriate metabolic capabilities. If these microorganisms are present, then optimal rates of growth and hydrocarbon biodegradation can be sustained by ensuring that adequate concentrations of nutrients and oxygen are present and that the pH is between 6 and 9 [24]. According to [8] and [13], mixed populations with overall broad enzymatic capacities are can degrade complex mixtures of hydrocarbons such as crude oil in soil. Bacteria are the most active agents in petroleum degradation, and they work as primary degraders of spilled oil in environment [24].

2.7 Bioaugmentation

Bioaugmentation is the introduction of a group of natural microbial strains or a genetically engineered variant to treat contaminated soil or water [18]. Usually the steps involve studying the indigenous varieties present in the location to determine if biostimulation is possible. If the indigenous variety do not have the metabolic capability to perform the remediation process, exogenous varieties with such sophisticated pathways are introduced [20]. Bioaugmentation is commonly used in municipal wastewater treatments to restart activated sludge bioreactors. At sites where soil and groundwater are contaminated with chlorinated ethenes, such as tetrachloroethylene and trichloroethylene, bioaugmentaion is used to ensure that the *in situ* microorganisms can completely degrade these contaminants to ethylene and chloride, which are non-toxic. Bioaugmentation could be typically performed in conjunction with the addition of electron donor (biostimulation) to achieve geochemical conditions in groundwater that favour the growth of the dechlorinating microorganisms in the bioaugmentation culture

2.8 Biostimulation

Biostimulation involves the modification of the environment to stimulate existing bacteria capable of bioremediation. This can be done by addition of various forms of rate-limiting nutrients and electron acceptors, such as phosphorus, nitrogen, oxygen, or carbon (eg. in the form of molasses) [25]. Biostimulation can be enhanced by bioaugmentation. The primary advantage of biostimulation is that bioremediation will be undertaken by already present native microorganisms that are well suited to the subsurface environment, and are well distributed spatially within the subsurface [18]. The

disadvantage is that the delivery of additives in a manner that allows the additives to be readily available to subsurface microorganisms is based on the local geology of the subsurface. Tight impermeable subsurface lithology (tight clays or other fine-grained material) make it difficult to spread additives throughout the affected area [20]. Fractures in the subsurface create preferential pathways in the subsurface which additives preferentially follow, preventing even distribution of additives.

2.9 Phytoremediation

Phytoremediation is the use of plants to depollute contaminated soils, water or air. It has been used to contain, degrade or eliminate metals, pesticides, solvents, explosives, crude oil and its derivatives, and various other contaminants from the medium that contain them [26] Phytoremediation is a process that uses actively growing plant roots to stimulate a diverse population of soil microorganisms, some of which have the capability to metabolize hydrocarbon contaminants. Fungi have also been successfully used to depollute petroleum contaminated sites. It is clean, efficient, inexpensive and non-environmentally disruptive, as opposed to processes that require excavation of soil. The principles of phytoremediation system include the uptake of dissolved nutrients and metals by the growing plants [27]. The most important factor in implementing phytoremediation is the selection of an appropriate plant [28,29], which should have high uptake of both organic and inorganic pollutants, grow well in polluted water and be easily controlled in quantitatively propagated dispersion [22]. Contamination arising from discharge of these wastes into the environment without treatment constitutes nuisance to the environment due to the persistent nature of the wastes and their tendency to spread into the ground and surface waters [24]. The ultimate goal of wastewater management is the protection of the environment in a manner commensurate with public health and socioeconomic concerns. Rahman et al. [30].

2.10 Application of Biological Methods

2.10.1 Isolation and identification of microbial strains

The crude oil contaminated soil samples are collected from different oil contaminated sites. The solvent extractable TPH in the crude oil contaminated soil samples are estimated [10, 31]. For enrichment, 5 g samples of soil are inoculated into 100 ml of minimal salt medium (MSM) [32] containing steam sterilized crude oil (1%, w/v) as carbon source and incubated at 37°C on a rotary shaker (200 rpm) for 7 days. Then 5 ml of enriched culture media is re-inoculated in fresh medium under similar conditions and five such cycles are repeated. After five cycles of enrichment, 1 mL of culture is diluted up to 10^8 fold, and 100μl of all dilutions are plated on MSM agar plates with crude oil (1% w/v). The bacterial colonies obtained are further purified on the MSM agar plates (with crude oil 1% w/v). The isolates are routinely subcultured and frozen stock cultures are stored in 25 % glycerol at -70°C. Identification of the isolated bacterial strains are done by sequencing of the 16S rDNA gene with the Microseq 16S DNA sequencing Kit TM (PE Applied Biosystems, Inc, USA) [33].

2.10.2 Degradation of crude oil by microbial strains

Degradation of crude oil by the bacterial isolates can be monitored in 250 ml flasks in triplicates containing 100 ml MSM with 1% (w/v) of crude oil (steam sterilized) as sole carbon source and incubation on a rotary shaker (200 rpm) at 37°C. The isolates are grown previously in MSM for 24 h with 1% (w/v) crude oil to a cell density of 10^8 cells ml^{-1} and are inoculated into the medium with 5% (v/v) as inoculum. Uninoculated controls are kept to monitor natural weathering of crude oil. Residual crude oil is extracted from the cultures by using solvents [34]. For quantitative analysis, the residual crude oil is fractionated by silica gel column [35]. The different fractions are analyzed by gas liquid chromatography (Hewlett Packard 5890 series II) fitted with flame ionization detectors [32]. The profile of the different fractions of petroleum hydrocarbons extracted from inoculated flasks is compared with that of the uninoculated controls to determine the extent of degradation.

2.10.3 Selection of microbial consortium

Based on the efficiency to degrade different fractions of total petroleum hydrocarbons (aliphatic, aromatic, asphaltanes, NSO compounds) and also based on the environmental parameters from where these bacterial strains have been isolated, few bacterial consortium can be developed for application on the actual field [32,33,36,37,38,39,40]. The crude oil degrading efficiency (qualitative and quantitative) of individual bacterial isolates is

usually screened on minimal salt medium using crude oil as sole carbon and energy source.

2.10.4 Selection and preparation of bioremediation sites

The type of contamination included acidic oily sludge and non-acidic waste oily sludge [41,42]. The bioremediation processes are carried out *ex situ* in different batches, where a secured HDPE (high density poly ethylene) lined bioremediation site is prepared near the sludge storage pit. The oily waste is excavated by using excavator and transported to the secured bioremediation site using dumper / trailer where the bioremediation process is executed. Walworth et al. [43].

2.11 Application of Microbial Consortium on Oily Waste

The microbial consortium is produced in 1500 liter bioreactor. The consortium is immobilized with a suitable carrier material, packed in sterilized polybags (packing size 5-20 kg) and transported to the respective sites for its application on oily waste. The consortium is applied on oily waste by manual spreading at regular intervals of one month. Specially designed nutrient formulation, containing Nitrogen (N), Phosphorous (P) and Potassium (K) compounds, is dissolved in water and spread uniformly to the bioremediation site with the help of water sprinkler. This is done to enhance the population of the microbial consortium and also to mitigate the initial toxic shock due to the oil contamination while application on the oily waste in the field. Mixing of oily waste and microbes is done by tilling of bioremediation soil sites. In the control site, microbial consortium is not added, however rest of the other activities like tilling, watering etc. is carried out in the same manner as the experimental bioremediation site.

2.11.1 Tilling and Watering

Tilling of the bioremediation sites is done at a regular interval of once in a week to maintain aeration for the microbial consortium at the bioremediation sites. This is done with the help of a tractor attached with cultivator or soil excavator. Watering of the bioremediation sites is done as per the requirement to maintain the moisture content of the soil for quicker biodegradation.

2.11.2 Sampling

Oily waste samples is collected from the bioremediation sites at zero day i.e. before

application of microbes on the bioremediation site and at every 30 days interval after application of the microbial consortium. The bioremediation site may be divided into four equal blocks, which is further divided in four sub-blocks. Equal quantity of samples are collected randomly from each sub-block i.e. total 16 samples are collected from one site. Samples are collected using a hollow stainless steel pipe of 3 inch diameter and 50 cm. in length and by inserting the same vertically on the bioremediation site from the surface till the bottom in one particular point. This is done to collect uniform samples from each depth of the bioremediation site. The samples are collected in sterile plastic containers.

2.11.3 Monitoring of Bioremediation process

- Characterization of oily waste
- Determination of Microbial count
- Determination of pH, Moisture content and selected heavy metals
- Biodegradation of TPH in the oily waste
- Toxicity studies

2.12 Factors Influencing Bioremediation of Oil Polluted Environment

Microorganisms have limits of tolerance for particular environmental conditions, as well as optimal conditions for pinnacle performance. Factors that affect success and rate of microbial biodegradation are nutrient availability, moisture content, pH, and temperature of the soil matrix. Inorganic nutrients including, but not limited to, nitrogen, and phosphorus are necessary for microbial activity and cell growth. It has been shown that "treating petroleum-contaminated soil with nitrogen can increase cell growth rate, decrease the microbial lag phase, help to maintain microbial populations at high activity levels, and increase the rate of hydrocarbon degradation" [40] However, it has also been shown that excessive amounts of nitrogen in soil cause microbial inhibition. [40] also suggest that maintaining nitrogen levels below 1800 mg nitrogen/kg H_2O and other nutrients would facilitate for optimal biodegradation of petroleum hydrocarbons.

All soil microorganisms require moisture for cell growth and function, availability of water affects diffusion of water and soluble nutrients into and out of microorganism cells. However, excess moisture, such as in saturated soil, is undesirable because it reduces the amount of available

oxygen for aerobic respiration. Anaerobic respiration, which produces less energy for microorganisms (than aerobic respiration) and slows the rate of biodegradation, becomes the predominant process. Soil moisture content "between 45 and 85 percent of the water-holding capacity (field capacity) of the soil or about 12 percent to 30 percent by weight" is optimal for petroleum hydrocarbon degradation [15]. Soil pH is important because most microbial species can survive only within a certain pH range. Furthermore, soil pH can affect availability of nutrients. Biodegradation of petroleum hydrocarbons is optimal at a pH 7 (neutral); the acceptable range is pH 6–8 [15].

Temperature influences rate of biodegradation by controlling rate of enzymatic reactions within microorganisms. Generally, "speed of enzymatic reactions in the cell approximately doubles for each 10°C rise in temperature" [11]. There is an upper limit to the temperature that microorganisms can withstand. Most bacteria found in soil, including many bacteria that degrade petroleum hydrocarbons, are mesophiles which have an optimum temperature ranging from 25°C to 45°C [11]. Thermophilic bacteria (those which survive and thrive at relatively high temperatures) which are normally found in hot springs and compost heaps exist indigenously in cool soil environments and can be activated to degrade hydrocarbons with an increase in temperature to 60°C. This however suggested an intrinsic potential for natural attenuation in cool soils through thermally enhanced bioremediation techniques [44].

Contaminants can adsorb to soil particles, rendering some contaminants unavailable to microorganisms for biodegradation. Thus, in some circumstances, bioavailability of contaminants depends not only on the nature of the contaminant but also on soil type. Hydrophobic contaminants, like petroleum hydrocarbons, have low solubility in water and tend to adsorb strongly in soil with high organic matter content. In such cases, surfactants are utilized as part of the bioremediation process to increase solubility and mobility of these contaminants [12]. Also, the existence of thermophilic bacteria in cool soil also suggest that high temperatures enhance the rate of biodegradation by increasing the bioavailability of contaminants. It is suggested that contaminants adsorbed to soil particles are mobilized and their solubility increased by high temperatures [44].

Soil type is an important consideration when determining the best suited bioremediation approach to a particular situation. *In situ* bioremediation refers to treatment of soil in place and this process involves bioventing, in which oxygen and/or nutrients are pumped through injection wells into the soil. It is imperative that oxygen and nutrients are distributed evenly throughout the contaminated soil. Soil texture directly affects the utility of bioventing, in as much as permeability of soil to air and water is a function of soil texture. Fine-textured soils like clays have low permeability, which prevents biovented oxygen and nutrients from dispersing throughout the soil. It is also difficult to control moisture content in fine textured soils because their smaller pores and high surface area allow it to retain water. Fine textured soils are slow to drain from water-saturated soil conditions, thus preventing oxygen from reaching soil microbes throughout the contaminated area [15]. Bioventing is well-suited for well-drained, medium, and coarse-textured soils.

3. CONCLUSION

One of the major environmental problems today is hydrocarbon contamination resulting from the activities related to the petrochemical industry. Accidental releases of petroleum products are of particular concern in the environment. Disposal methods such as incineration or Mechanical and chemical methods generally used to remove hydrocarbons from contaminated sites have limited effectiveness and can be expensive. Bioremediation is the promising technology for the treatment of these contaminated sites since it is cost-effective and will lead to complete mineralization. Bioremediation functions basically on biodegradation, which may involve complete mineralization of organic contaminants into carbon dioxide, water, inorganic compounds, and cell protein or transformation of complex organic contaminants to other simpler organic compounds by biological agents like microorganisms. The mechanism of biodegradation has a high ecological significance that depends on the indigenous microorganisms to transform or mineralize the organic contaminants. Microbial degradation process aids the elimination of spilled oil from the environment after critical removal of large amounts of the oil by various physical and chemical methods. This is possible because microorganisms have enzyme systems to degrade and utilize different hydrocarbons as a source of carbon and energy.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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