CLEAN-UP OF CRUDE OIL CONTAMINATED SOILS: BIOREMEDIATION OPTION –A REVIEW

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Abstract: As the world moves toward "a nontoxic environment" there is an increase action in soil and ground water remediation activities in response to the world's environmental quality objectives. Crude oil is a pollutant whose entrance into the soil and water environment has elicited profound negative impacts on the environment as a harbinger of soil, water and air pollution. The impacts is unprecedented and cannot be overlooked and as such they need to be removed from the environment after a spill as they are potent immunotoxicants and carcinogenic which can led to kidney diseases, cancer and liver damage. Bioremediation, a technology that exploits the various degradative abilities of microorganisms in the environment to convert organic contaminants to non-toxic products such CO_2 and water by mineralization has become the process of choice in contaminant removal. The bioremediation technology is deemed efficient, low cost, requires little or no technical knowledge to operate, and has no overall negative consequence on the environment. Although the effectiveness of the bioremediation system is constrained by the properties of the contaminants, the soil matrix as well as the environmental factors, it is still the process of choice for environmentalist. This article provides a review of bioremediation process highlighting the different strategies needed to accelerate the process and showing that optimizing the environmental factors can influence the bioremediation process.

Keywords — Biostimulation; Crude oil; Nutrients; Remediation; Response Surface Methodology; Soil Matrix.

1. INTRODUCTION & BACKGROUND

The growth of industries and man's total reliance on fossil fuel as a primary source of energy has led to a wide spread exploitation, exploration, refining and transportation of crude oil. Crude oil derived products are a major source of energy for homes and industries and their entrance into the environments either by accident or sheer negligence comes with adverse effect on humans, plants and animal health as the contaminants are carcinogenic [1, 2]. The preponderance of environmental pollution occasioned by crude oil exploitation and usage is a worldwide problem and has generated the need to search for an environmental low cost clean-up technique for removal or reduction [3]. The total estimated sites of crude oil contamination around the world are significant [3, 4] and has resulted in the alteration of ecological balance in the contaminated ecosystem and there is need to effect immediate cleanup of such sites. Though crude oil is not classified as a hazardous chemical, it is the commonest environmental contaminant and their presence in the environment has elicited different analytical and remediation methods [5, 6].
The conventional method of remediation is the traditional removal of contaminated soil to a landfill. This method is replete with drawbacks and consequently a better technology that will completely destroy the pollutant or transform them to a harmless product was sought.

Numerous investigation on the technology of remediation for soils and other ecosystems contaminated by crude oil spillage, is ongoing and major conclusions have been drawn from such investigation with attendant technologies including bioremediation [7], phytoremediation [8, 9], methods involving chemical decomposition and chemical oxidation [10], Other technologies have also been put to use which included the high-temperature incineration model as thermal remediation [11]. Also, solvent, super critical fluid and ultrasonic extraction [12], are all crude oil impacted soils remediation methods that are increasingly being put to use.
Nonetheless despite the effort, most of this methods of removal are technological complex and extremely costly and lack public acceptance [3]. Bioremediation on the other hand is a cost-effective and a promising biotechnology approach, increasingly being studied and implemented, offers the possibility to destroy or render harmless various contaminants including petroleum hydrocarbon using natural biological activity, has advantage over other methods to detoxify or degrade environmental pollutants.
It is a controlled process of organic substances degradation that relies on the ability bacteria or fungi to degrade or transform hazardous contaminants to materials such as carbon dioxide, water, inorganic salts, microbial biomass, and other byproducts that are less hazardous than the parent materials [13, 14]. The use of microbes to decontaminate crude oil impacted soils is adjudged to be efficient and effective and an alternative to the traditional methods. Although the cost of bioremediation treatment is enormous, this huge operating cost is compensated by reduction in clean up time. And unlike the traditional methods that transfer contaminants from one medium to another bioremediation destroys contaminants completely by converting them to CO_2 and water.

Though there are several treatment methods that exists to remediate polluted sites, selecting an appropriate site specific remediation technology is very challenging. The issue of approach in the selection of remediation technologies for soil cleanup was addressed by looking at the physical, biological and chemical processes encountered in soil remediation. Ronald and Judith proposed a conceptual approach based on information employed at waste sites which includes identifying, quantifying and controlling contaminants sources and considering the cleanup required for the soil medium to protect human health and environment [15].

2. Crude Oil as a Soil Contaminant

Crude oil and products are recognized as a major contributor to health and environmental hazard especially in areas of intense human activities. The frequency and degree of soil contamination by crude oil and petroleum products is a pervasive problem that is universally felt and the consequences are extremely high and a dangerous threat to human, animals and plant health [16]. Crude Oil contamination in soil can affect the soil physical and chemical properties such as the maximum surface temperature [17]. The entrance of crude oil in soil makes the environment, anaerobic by blocking the diffusion of air, which affects the soil microbial communities [18, 19, 20]. The aromatic hydrocarbons, benzene, toluene, ethylbenzene, and xylene (BTEX) are compounds with one or more fused aromatic rings found in crude oil. Their entrance into the environment gives much concern as they are carcinogenic or may be converted into carcinogens by microbial actions when crude oil is spilled. Crude oil in soil cause complete mortality of mangrove vegetation [21], inhibits seed germination by creating nutrient deficiency, which may lead to stunted plant growth or death on contact.

Crude oil spilled on land prevents water absorption by the soil; and spills on grassland and agricultural land have the effect of choking off plant life. Spilled hydrocarbons could be held in voids in the soil forming a large bank of residual saturation which may lead to a long contamination of ground water if not removed [22].crude oil in soil can also increase the soil total organic carbon [23], and change soil pH values [24].

The level of soil contamination and remediation measures taken, will determine how long the impacted soils will remain unsuitable for crop growth. The sustainability of the soil is of an immense interest and concern to us because of the direct reliance of our existence on it. It is therefore essential that soil quality, fertility and productivity should be continually maintained and monitored. Crude oil contaminated sites represent a major challenge in many communities in the Niger Delta area of Nigeria. Local soil contamination and ground water pollution are majorly associated with the operations and activities of the oil industry.

3. Fate of Crude Oil in the Soil

Several studies have examined the fate of hydrocarbon in the soil environment and other ecosystems. Crude oil is recognized as a substrate that supports microbial growth and it is both a target and product of microbial activities [25, 26]. When crude oil enters the environment, it is subject to many physical, chemical and biological changes that contribute to its loss or alteration [27]. If conditions for biodegradation are ideal, the hydrocarbon could be completely mineralized to innocuous products. Portion of the crude oil mass will volatilize and some portions will solubilize as components of soil vapour and groundwater. The volatilization and solubilization tends to make the remaining mass of crude oil denser, less mobile and more difficult to biodegrade [28]. The partially degraded hydrocarbon is incorporated into the soil as part of the organic matter [29]. Such physical, chemical and biological changes also include biodegradation, evaporation, adsorption, penetration, migration and release. These weathering processes alters the properties of the crude oil in such a way as to affects the method of removal from the environment. According to Atlas RM, "microorganisms in the ecosystem have the ability to utilize the hydrocarbons as the sole source of carbon and energy and such microorganisms are widely distributed in nature" [30]. It is extremely important that the microbes and the contaminants be in contact and therefore the bioavailability of the substrates to the degrading microbial communities is essential for maximum remediation of the ecosystem [3].

The degree to which petroleum hydrocarbons are retained in the soil is determined by the properties of the oil, which includes the oils structural complexity as well as the soil environmental conditions. Other important crude oil properties that affect degradability include the API gravity and viscosity. The soil environmental factors that limit the rate of hydrocarbon biodegradation include temperature, soil pH, moisture content, soil texture, sorption, bioavailability, salinity, contaminant concentration and the presence of microbial toxins [30]. Other factors that affect biodegradation include chemical composition of the crude, volume of the released crude oil, its physical state, volatility, pH, Biochemical Oxygen Demand (BOD) etc. The behavior of crude oil pollutant in the environment also depends upon a variety of other processes and properties.

1. Chemical processes, e.g. hydrolysis, oxidation and reduction.
2. Physical or transport processes and properties, e.g. advection, evaporation, leaching, dispersion and diffusion, volatilization.
3. Biological processes, e.g.: bioaccumulation, biotransformation, biodegradation, and toxicity and
4. Combined environmental factors.

4. Bioremediation as a Cleanup Technology

As the need to search for new environmentally friendly technologies to cleanup pollutant arose, bioremediation was considered as a promising alternative to other systems in use. Different methods of removal of crude oil products from the environment abounds but presently employing the biological treatment of bioremediation is the most potent, popular and cost effective strategy [31]. Bioremediation is a technology based on the science of biodegradation which is environmentally acceptable, and effective remediation method that exploits the various degradative abilities of microorganisms in the environment to convert organic contaminants to non-toxic products such CO_2 and water by mineralization [32]. Bioremediation provides a complete transformation or removal of organic compound even at low concentration. Bioremediation remains the method of choice in the quest to clean up the environment contaminated by crude oil and is rather considered as a permanent solution than a remediation method. According to Bartha the microbiological decontamination of oil contaminated environment is assessed to be efficient, economical and versatile alternative to physicochemical treatment [33]. Sometimes bioremediation may be employed in order to attack specific contaminants, as chlorinated pesticides that are degraded by bacteria, or oil spills that are broken down using multiple techniques including the addition of biosurfactant to facilitate the crude oil decomposition by bacteria [34, 35]. Enhanced bioremediation, a process in which indigenous or inoculated microorganism degrade organic compounds encompasses a range of technologies that differ with respect to their inputs [36]. Accord to Vidali, "the science of bioremediation is not complex, but it requires considerable measure of experience and expertise to design and implement a successful bioremediation program" [3]. Therefore advances in science and engineering are necessary and essential to manipulate, design and use different input parameters to enhance the rate and degree of biodegradation.

Studies show that the effectiveness of a bioremediation system is constrained by the properties of the contaminants, the soil matrix as well as environmental factors [37]. Therefore the evaluation and selection of a bioremediation strategy for the effective remediation of crude oil contaminated soils system will require a careful consideration of the contaminated sites as well as the soil factors and characteristics [38]. The soil matrix has a considerable influence on the removal of hydrocarbon from soil. To achieve optimum biodegradation there is need to optimize the soil parameters and reduce the treatment time of the bioremediation systems by accelerating the rate achieved through employing a wide variety of technologies. It also involves a good selection of the biostimulation strategy and proper assessment of the influence of environmental parameters on the rate of degradation and the optimization of these factors for maximum petroleum hydrocarbon removal by employing an effective statistical approach. Bioremediation techniques has been successfully used to remediate soils and ground water contaminated with petroleum hydrocarbon, pesticides etc.

There are numerous crude oil contaminated sites all over the world with the Niger Delta oil producing communities in Nigeria harbouring a substantial number of these sites. Adequate knowledge on what constituted the operative factors in bioremediation process is necessary in making decision to assist the biodegradation efficacy in crude oil impacted soils.

5. BIOREMEDIATION TECHNOLOGIES FOR CRUDE OIL REMOVAL FROM THE SOIL

The need to adopt effective remediation techniques led to the development of several physical, chemical, thermal and biological technologies [39]. Technologies commonly employed in soil remediation include mechanical, burying, evaporation, dispersion and washing [40, 41]. These technologies have limited effectiveness as they rarely achieve complete cleanup of oil spills and generally, they are expensive and can lead to incomplete decomposition of contaminants [3]. They only transfer the contaminants from one environmental compartment to another [27]. And because of the limitations of the physiochemical methods, there is this need to develop technologies that would take care of the inadequacies of the aforementioned techniques. Great deal of literature has reported that bioremediation technologies are alternative to the methods. Bioremediation is the most popular and cost – effective biotechnology strategy that is increasingly being studied and implemented among the other methods, to detoxify or degrade crude oil and other environmental pollutants from the soil to harmless substances. It is an enhancement of the natural fate of hydrocarbon pollutant with little or no attendant negative environmental effect [42].

Bioremediation techniques are essentially destructive technique, easily implemented at low cost, effectively inexpensive [27, 43] and geared toward simulating the growth of microorganisms that uses the crude oil contaminants as food and energy source by creating favourable environments for the microorganisms to thrive. Biodegradation of crude oil contaminant by natural population of microorganisms in soil represent one of the primary mechanism by which crude oil can be eliminated from the soil environment and is governed by physico-chemical factors (e.g. nutrient, pH, temperature, moisture content) as well as the soil factors.

Das and Mukherjee and many others researchers have reviewed extensively the requirements for optimal microbial growth under a variety of circumstances, and the degradation trails for crude oil have equally been detailed by other researchers [40, 44, 45]. Furthermore, the influence of soil parameters and crude oil physical interactions such as mass transport, sorption and desorption [46] on the remediation rate is also well documented in literature too. A variety of techniques for environmental remediation have been compiled and summarized as shown in tables 6-1 and 6-2.

6. BIOREMEDIATION STRATEGIES

The objective of bioremediation treatment is to degrade contaminants to an innocuous species. Bioremediation utilizes the natural role of microorganism in the contaminated media to transform or mineralize inorganic contaminants to a level where they will no longer put human, animals or plant in a harm way. Over the years several treatment strategies have been proposed and developed for treating petroleum hydrocarbon contaminated sites and a number of new and promising approaches are under development. The primary technique in bioremediation involves the enhancement of the activities of microorganism through the addition of nutrients, controlling the environmental parameters or through the addition of external microorganisms (bioaugumentation) [47, 48]. These treatment methods are classified into two basic categories: *in-situ* and *ex-situ* treatment based on the place where the contaminated materials are treated, Ex-situ technologies refer to treatments that remove contaminants to a separate treatment facility, while in-situ bioremediation technologies is the term used for treatments of contaminants in the place itself and it is considered to minimize material handling and reduction in costs [49]. This techniques are categorized as biological, chemical, or physical, are covered for contaminated soils and environmental waters.

6.1. In Situ Bioremediation Strategy

In situ or on-site strategy is defined as those techniques that are applied to soil and groundwater at the site of pollution where the soil is not unearthed but treated in the original place of contamination with minimal disturbance. Application of the in-situ technique is dependent on the availability of oxygen and the penetration depth of the hydrocarbon into the soil and on the nature of the soil if it is groundwater saturated or unsaturated and if the contaminants in the subsoil are biodegradable. If contaminants are recalcitrant bioaugmentation with adapted or specially designed microbial inoculants is a useful alternative [50]. Adopting the in-situ technology option is good as excavation and transportation of contaminated materials is avoided but achieving uniform remediation is challenging because of soil heterogeneity [51]. Technologies of note in in-situ bioremediation are summarized in table 6.1.

Table 6-1: In-Situ Technologies

S/No	Bioremediation process	References
1	Natural Attenuation	[52, 53, 54]
2	Biostimulation	[45]
3	Bioventing	[55]
4	Biosurfactants	[22]
5	Bioaugumentation	
6	Phytoremediation	[56, 57]
7	Electrokinetic Separation	[58]
8	Heating	[11]
9	Soil Vapor Extraction	

Emerging techniques that could be used in the remediation of crude oil contaminated soils include microbial fuel cells, nano-remediation, genetic engineering, and photo-hetero microbial system.

6.2. Ex Situ Bioremediation Strategy

In ex - situ technology approach, impacted media are physically excavated or pumped from the contaminated site to another location for treatment and subsequently returned to the site after treatment in record time. If the groundwater is found to be contaminated it is also removed along with the soil for treatment. Advantages include the uniformity of treatment, easy monitoring and the possibility of screening and homogenization of the contaminants [59]. But the cost of excavation and transport is high. Existing Ex-Situ remedial options for contaminated soil includes: Dig-and-Dump (Landfills and Engineered Landfills), Pump and treat, Incineration, Oxidation, Adsorption, Iron Exchange, Soil Washing [60], Pyrolysis

Table 6-2: Ex-Situ Technologies

S/No	Bioremediation process	References
1	Land Farming	[61, 62]
2	Composting	
3	Bioreactors	
4	Bioslurry Reactors	
5	Bioslurping	[63]
6	Soil Washing	[60]
7	Biopile	[64]

In most cases the physico-chemical and biological technologies is integrated for better cleanup of polluted sites.

7. Bioremediation as a Process of Crude Oil Removal from the Soil Environment

Bioremediation defined as "a grouping of technologies that uses bacteria or fungi to degrade or transform hazardous contaminants to materials such as carbon dioxide, water, inorganic salts, microbial biomass, and other byproducts that may be less hazardous than the parent materials" [13, 14]. Whereas biodegradation refers to a controlled process in which organic substances are broken down by the actions of bacteria and fungi to other less hazardous substances such as carbon dioxide and water. Bioremediation on the other hand is much about the restoration of contaminated environment through the actions of bacteria or fungi to degrade, remove, immobilize or alter a contaminants and this could be seen from the standpoint of biodegradation. Biodegradation, described by Hoff as "a component of oil weathering and is a natural process whereby bacteria or other microorganisms alter and break down organic molecules into other substances, eventually producing fatty acids and carbon dioxide" [65]. Bioremediation is the hastening of this process of biodegradation by the addition of external microbial populations that is not indigenous to the soil [50], through the biostimulation of indigenous bacteria populations by the addition of organic and inorganic nutrients [66], also by manipulating environmental media such as soil pH, temperature, soil moisture content and aeration, the bioremediation process could be enhanced. [30]. Bioremediation technologies abound and it includes such technologies as biostimulation, bioaugumentation, bioventing, bioreactor, phytoremediation, composting etc.

Bioremediation will not always be a win all solution in all remediation events; it is limited by the types of contaminant it could be used for and it is not regarded as a mature technology. Although it is not doubtful that bioremediation has great potential in dealing with crude oil contaminant, however, the length of time needed to remove the contaminants completely from a contaminated environment is long, and the degree of contaminant removal levels achievable may not always meet the standard desired by the regulating authorities [67].

8. Factors Affecting the Bioremediation of Crude Oil Contaminated Soil

In the effort to harness the ability of microorganisms to get rid of crude oil from a contaminated environment, a careful selection of microorganisms and the sustenance of such optimal soil conditions that are necessary and favorable to their growth, are imperative [68]. The optimal rate of crude oil biodegradation could be achieved and sustained by ensuring that all the factors that favours rapid crude oil degradation such as the physical and chemical parameters like the pH of the soil, temperature, soil moisture, soil nutrients and available water content [30, 69] must be maintained.

Studies by various remediation practitioners shows that high molecular weight, aromatic and branched hydrocarbons are not easily degradable as compared to the lighter, straight chained and saturated hydrocarbons. Other authors have demonstrated similarly that application of agricultural methods such as tilling, sprinkling with water and addition of organic manure as cow dung straw, pig manure and inorganic fertilizer could be used to decrease the contamination level of crude oil in the environment [70]. Inspite of this, many environmental restrictive factors have been acknowledged to affect the rate of removal of crude oil contaminants in soils and the most important of these parameters are gradually being optimized in the effort to ensure a cleaner environment.

These factors include microorganism type, nutrients availability, soil pH, temperature, moisture content of the soil, oxygen availability, other soil properties, and the contaminant concentration [71, 30]. The environmental parameters do not affect the rate of bioremediation separately but they interact and the rate of biodegradation usually responds to the most limiting factor [27]. Many contributors on this topic are of the opinion that the disappearance of crude oil from the contaminated environment is mostly accelerated by the addition of nutrients, like nitrogen or phosphorus or both. Therefore it can be conclusively said that the main requirements for a successful biodegradation process are an energy source in form of nutrient and a carbon source in form of crude oil. In summary, (a) The intrinsic ability of the microorganisms at the site, (b) Characteristics of the crude oil (c) Availability of nutrient and the environmental factors are the major factors limiting the biodegradation of hydrocarbon contaminants in soil.

9. EFFECT OF SOIL TEXTURE ON CRUDE OIL REMEDIATION

The soil is known to be a habitat to crude oil degrading microorganisms and the soil characteristic is important in the degradation of crude oil. The soil is classified into four distinct categories as coarse material, sand, clay and silt and they contains moisture and air. The matrix of the soil influences the removal of petroleum hydrocarbon from a crude oil contaminated environment. Some characteristics of the soil that determines how favourable a microbial degradation process will proceed includes, texture, permeability, pH, nutrients, water holding capacity and availability of oxygen. Pore spaces in soil are also important and the amount of pore spaces in the soil is dependent on the soil type and degree of compaction. Clay soils generally have a higher degree of pore spaces than sandy soil but may not allow the delivery of nutrients in an efficient manner because of the physical size of the pores. Regularly large quantities of spilled oil could be held in voids in the soil forming a large bank of residual saturation which could lead to the continual contamination of groundwater if not removed on time, The soil type, the sorptive surfaces, available soil organic matter and intrinsic bioavailability of the crude oil fraction, is the most important consideration in the appraisal of the suitability of a bioremediation method. A particular type of soil that hardly received mention in the issue of remediation of crude oil impacted soils is the mangrove soil which incidentally is the soil of type in most part of the Niger delta where crude oil spill is at epic proportion. A valuation of the mangrove soil parameters vis-a-vis the effect on the rate contaminant degradation is important. Generally it is acknowledged that the mangrove soil is rich in features that aid bioremediation such as clay, organic matters, minerals and exchangeable bases than the non-mangrove soils [72]. Another feature of this soil is the high salinity, a factor that must be incorporated into the rate equation for mangrove soil contaminated with crude oil. When contaminants are released in the soil matrix they migrate to the soil macropores and are adsorbed to the surfaces of the medium aggregates. It then travel further, and migrate into the micropores, which are narrow, inaccessible apertures and surrounded by the soil particles. For that reason, clay generally has negative impact on the degradability of hydrocarbon in soil. Indeed, reductions in particle sizes of clay, increase the specific surface area, and thus add to the entrapment of contaminants in tight spaces.

10. BIOSTIMULATION

One of the results of bench scale studies of crude oil polluted environment remediation is biostimulation which involves the manipulation of abiotic factors to optimize conditions necessary for indigenous microbial remediation of contaminants or stimulation of the degrading abilities of microorganisms by the addition of rate enhancing nutrients. According to Venosa et al. the major objective of laboratory biostimulation studies is to evaluate the types, concentration, and frequency of addition of nutrients amendments necessary to encourage the growth and activities of naturally occurring microorganisms in field environment for maximum stimulation [73]. This option is only adopted when there are indigenous microorganisms with biodegradation ability but the rate is slow and needed to be beefed up.

Other studies on the biostimulation experiments indicates that addition of inorganic nutrients such a nitrate and phosphate, electron acceptors and other substrates as organic manures has greatly enhanced the rate of crude oil removal from the soil environment. This suggests that nutrient supplementation stimulates bioremediation by increasing microbial biomass.

However, the optimal nutrient concentrations and types necessary for effective bioremediation of contaminants vary greatly with respect to the properties of the hydrocarbon and the environmental conditions and the ability to maintain this optimal level in contact with the soil is a difficult issue [27].

For biostimulation to be effective in the amelioration of crude oil contaminated soil the concentration of nutrient in the environment must be considered and this concentration must be maintained at optimal level in order to stimulate the growth of the microbes. It should be noted that excessive concentration of nutrient in the ecosystem environment could induce toxic response as well [27].

11. AMENDMENTS NECESSARY FOR EFFECTIVE BIOREMEDIATION APPLICATION

The effectiveness of bioremediation technology is highly affected by the soil environmental characteristics highly related to the types of soil, soil being the medium in which the remediation will take place places emphasis on the soil parameters as a necessity that must be evaluated. As implied earlier on, that the success of bioremediation technology application to crude oil contaminated soils requires knowledge of the characteristics of the site as well as the site constraints that hinders the microbial biodegradation of pollutants [74]. However, the correct optimization of these soil parameters must be taken into consideration. Several soil factors have significant effects on the biodegradation of crude oil contaminants and these factors include nutrients, water content, temperature, and soil pH. The biodegradation of contaminants in the soil can therefore be enhanced by making these environmental factors optimum for the required reactions.

11.1. Soil pH

The pH of the soil has an effect on the biodegradability of hydrocarbons pollutants and is highly variable ranging from 2.5 to as high as 11. The suitability of a pH range for any bioremediation work is site specific and is influenced by the complex relationship between the organism, the contaminants and the properties of the soil environment. The soil pH affects the solubility and consequently the availability of many constituents of the soil and it is highly variable over a wide range. The optimal microbial activity is improved when pH is close to 8. At this range the fungi is more tolerant of the condition than bacteria [30]. Studies by Robert Riser shows that the optimum pH for rapid decomposition of contaminants in soil is normally in the range of pH 6.5 to 8.5 [46]. Dibble and Bartha in their work concluded that a soil pH of 7.8 was nearly optimum [66]. Soil pH affects permeability, influences the dissolution of soil metals, the growth of microorganisms and determines nutrients accessibility [75]. The soil pH is continually monitored for deviations from optimum and subsequently adjusted by adding chemical reagents. If the soil is acidic, agriculture lime is employed to raise the pH; on the other hand if the pH is high aluminum sulfate or sulfur could be used to bring down the pH to required optimum.

11.2. Soil Moisture Content

Soil moisture is an important parameter in bioremediation. The estimation of moisture content and the maintenance at ultimate level is critical in the study of the remediation of crude oil impacted soil. The amount of water that a soil holds is assessed during soil characterization and it influences the effectiveness of bioremediation technology by controlling the movement of air [75]. Water is the medium through which nutrients and organic constituents pass into the microbial cell and waste products pass out of the cell [76]. The microorganisms involved in the remediation process are more active at optimum moisture content of the soil. Water in excess of the maximum or below the minimum can be unfavorable to an aerobic bioremediation operation [77]. If the contaminated soil is saturated with water the passage of oxygen will be inhibited, on the other hand in a situation of dry conditions microbial activity will slow down or halt the biodegradation process. Luo et al. concluded that the desirable range that will permit the passage of oxygen for microbial respiration and water conditions to between 70 to 80% of soil's water holding capacity. According to Ben Banipaul "a soil is said to be at field capacity when soil micropores are filled with water which facilitates the diffusion of soluble substrate and macropores are filled with air which makes O_2 diffusion easier" [75] and the water holding capacity is dependent on the nature of the soil. Table 1 culled from the work of Ben Banipaul provides a general soil moisture characteristic for two types of soils. If the moisture content of the field is maintained at optimum levels, studies by Ben Banipaul shows that generally clay soil biodegradation rates are higher than sandy soil.

Table 11-1: soil characteristics for effective bioremediation treatment [75]

Soil type	Moisture holding capacity	Permeability	Field capacity	Wilting point
Sandy	High	Low	9-25 %	3-10 %
Clayey	Low	High	38-43 %	25-28 %

11.3. Oxygen Supply

The presence of oxygen is an important and necessary condition for biodegradation to occur as microorganism employs 3-4 parts of dissolved oxygen to oxidize one part of hydrocarbon to water and carbon dioxide. Although degradation can occur in the absence of oxygen but in the aerobic treatment of hydrocarbon contaminants in soil the supply of oxygen is limited as the soil get saturated with water above the optimum and oxygen is consumed faster than it can be replaced resulting in an anaerobic condition. Tilling may be applied as an effective means of aeration to enhance the supply of oxygen and accelerate the crude oil removal from a contaminated soil environment.

12. MONITORING CRUDE OIL BIODEGRADATION

Massive oil spill has prompted increased research on techniques available to monitor crude oil degradation, however, efforts to verify biodegradation processes are limited by methodology [78, 79]. The traditional method of monitoring biodegradation relies on sampling and analysis but this method is replete with errors. Consequently attention is gradually shifting to the geophysical method due to their noninvasive nature, spectral and cost effectiveness [80]. One of such method is the spectral induced polarization method (SIP). In the soil and marine environment, the Spectral Induced Polarization (SIP) method is sensitive to biogeochemical changes occurring because of microbial oil degradation. In the traditional method the rate at which the crude oil pollutant is removed from the soil environment may be indirectly measured by the respirometric techniques [38, 77], where the carbon dioxide or oxygen formed from microbial respiration is taken as a measure of the degree of removal of the crude oil pollutant from the soil. Most researchers engaged in bench scale laboratory studies uses the carbon dioxide measurement to determine the rate and magnitude of biodegradation. In the application of this technique a respirometer with oxygen and carbon dioxide (CO_2) sensors and biometer flasks is used to measure microbial respiration rates of soil samples. Respirometry is a proven technique in the determination of biodegradation rate in water but it is yet to be proven in the soil [43].

Another method that serves as a measure of evaluating the level of remediation that has been achieved is by following the concentrations of particular oil constituents in the spilled environment. The components of crude oil degrade at different rates, with the lighter hydrocarbon easily degraded even by abiotic processes leaving the heavier constituents such as the cycloalkane which are resistant to attack by microorganism to persist in the soil. The overall degradation rate of oil is not feasible but we can use the method of TPH a term used to describe a mixture of chemical originally from crude oil [81] to evaluate the crude oil contamination of soil resulting in a sum parameter that does not give the concentration of any specific compound. The TPH measurement is performed to determine the total amount of hydrocarbon present in a given environment. Analysis using the Total Petroleum Hydrocarbon (TPH) to establish the degradation rate and evaluate the degree of disappearance of the oil contaminants from the polluted soil does not provide information on the composition of the oil but it is the most preferred method by most researchers and organizations. The values steams from conventional analytical methods and is connected with environmental sampling. The TPH technique includes the gravimetric gas chromatography/flame ionization detection (GC/FID) and infra-red (IR) spectroscopic methods. Other methods include gas chromatography/mass spectrometry (GC/MS) and thin layer flame ionization (TLC/FID) methods [27].

13. Rate of remediation (Kinetics of crude oil biodegradation)

For soils contaminated by crude oil in which the biodegradability of the oil is unknown, it is basic to undertake a laboratory investigation of biodegradation kinetics of that soil contaminant in order to determine the rate and duration of bioremediation treatment. The rate and the extent to which microorganisms will removes organic compounds from the soil could be expressed mathematically to estimate the time for remediation. According to Yelebe *et al*. whose opinion were that even when much work may have been done in bioremediation studies and practice, yet very little is known of the kinetics of bioremediation and how it affects the performance of several available bioremediation options [82] including the effect of soil heterogeneity. Zhu et al concluded that a handy information on the kinetics of crude oil biodegradation under different environmental conditions was imperative for evaluating the potential fate of targeted pollutants, evaluating the effectiveness of bioremediation, and determining appropriate strategies necessary to enhance crude oil biodegradation [27].

So in the quest to find out the rate of degradation of contaminants in the environment, it is appropriate that the minimum set of factors or variables that are necessary requirements for the rate of degradation determination be included in the rate equation.

The soil factors, nutrients concentration, soil moisture content, soil pH, and temperature are the likely key parameters that should be introduced into most of the models to predict the rates of crude oil removal in the environment. There are so many rate studies of crude oil biodegradation rate conducted under laboratory conditions but very few kinetic studies under field conditions. Yelebe *et al* (2015), working on a similar process and relying on certain assumptions developed some models that was fitted to experimental data for nutrients enhanced bioremediation [82]. The models they considered included scenarios where (a) the microbial growth was exponential and yield constant, (b) microbial growth was exponential but yield was not constant and (c) growth is logistic and yield is constant. Song et al. developed a base model based on CO_2 accumulation data as:

$$V_c(t) = \alpha t^b \quad \text{.........................} \; (1)$$

Where V_c is the cumulative volume (µl) of CO_2 produced through microbial respiration, t is the incubation time (hour), and a and b are coefficients that are functions of various physical and chemical parameters. And differentiating equation 1 to obtain the bioremediation rate at any point in time as [38]:

$$R(t) = V_c(t) = abt^{b-a} \text{.......................}(2)$$

Other investigators on the Exxon Valdez program developed a rate of remediation model from multiple regression analysis for remediation field studies as shown in equation 3 [27].

$$C_h(t) = \alpha[1 - p(t)]^{\gamma} e^{\delta r(t) + \omega t} \varepsilon \; \text{.......} \; (3)$$

Where:

C_h (t) = concentration of the analyte.

p = polar fraction of the oil

r = ratio of residual nitrogen concentration to oil,

ε = assumed multiplicative error term

α, δ, γ, and ω = multiple regression analysis parameters [27].

Venosa et al. (1996) also compared oil biodegradation rate obtained from bench scale laboratory studies with that of the field studies and concluded that the rate of the targeted component degradation in the field was lower than that of the laboratory. The rate equation representing first order degradation rate constant for the alkanes and three ring PAH hydrocarbons in crude oil is expressed as:

$$\left[\frac{A}{H}\right] = \left[\frac{A}{H}\right]_0 e^{-kt} \quad \text{.................................} \ (4)$$

Where:
(A/H) = analyte hopane-normalized concentration,
$(A/H)_0$ = analyte quantity at T=0,
k =1st order rate constant.

14. Statistical analysis (establishing the optimal parameter levels for biodegradation)

Numerous authors including Paveen et al, Kumar et al, [83, 84] and so many others have proposed different mathematical models based on statistical analysis to predict the migration or fate of crude oil contaminant in soils; however most of these models fail to include the complete set of soil parameters nor consider the effect of soil heterogeneity and texture that are closely associated with microbial activities. Most presume that biodegradation to be a simple first order process and propose equations to treat it as such. Some investigators Seyed Foad Aghamiri et al. employed a design of experiment (DOE) technique based on the Taguchi method to optimize the rate of hydrocarbon removal from soils and obtained a set of optimum conditions for the 8 factors investigated and about 68% of crude oil removed after 20 days experimentation.

Another researcher, Agarry, (2018) using the Box-Behnken model of Response Surface optimization tool studied the effects of inorganic and organic nutrients and activated carbon on the rate of degradation of weathered crude oil. He concluded that biostimulation using inorganic fertilizer and activated carbon as biostimulants resulted in significant enhancement of the degradation rate of crude oil in soil [32]. Golam Taki also employed the Box-Behnken design method of Response Surface Methodology to optimize some set of operating parameters in order to remove and recover crude oil from contaminated soil using subcritical water extraction process and attested to the usefulness of the Box-Behnken Design method.

The effects of soil factors and soil heterogeneity, a major factor in bioremediation studies was never considered in any of this work and therefore their efforts towards the establishment of credible relations to predict the rate and degree of removal of crude oil from soils may not accurately predict the rate of remediation in the field. It is known that soil heterogeneity results in significant variation in degradation rate and makes it more difficult to extrapolate the rate from laboratory studies to field studies [38]. For a laboratory scale study to be significant soil heterogeneity and texture must be incorporated into the rate equation.

According to works of Sturman P et al and Song Jin *et al.*, results and correlations developed in a laboratory setting do not always accurately represent what will happen in the field, as there are variations caused by soil heterogeneity, pH, moisture content, temperature and aging of contaminants [85, 38]. Holcomb and Pigott concluded that in all remedial scenarios, corrective action plans using bioremediation should be evaluated on a site-by-site basis because microbial action is controlled by site conditions [86]. The need to relate the effects of soil types to the bioremediation rate of contaminants in soils are imperative and those conditions affecting it must be optimized [69]. The accurate measurement of biodegradation rates is necessary and essential for successful, cost effective application of bioremediation method in pollutant removal from contaminated media.

15. Conclusion

As the world heads toward “a nontoxic environment” there is an increase action in soil and ground water remediation activities in response to the world’s environmental quality objectives.

Significant evolution in bioremediation practices, a multi-disciplinary perspective based on integrated scientific principles has been witnessed over the years focusing primarily on the impact of hazardous chemicals on the environment and human health and the end product of a bioremediation treatment is usually a harmless products such carbon dioxide and water. Bioremediation of petroleum, contaminated soils is a viable and cost-effective technology and the ease of application on crude oil contaminated soils make it more efficient than many other conventional methods resulting in considerable savings in costs.

Although the technology is simple and economical, easily deployed and managed but efforts to extrapolate information on rates from laboratory and bench scale studies with field scale practices are hampered by tremendous diversity in measurement techniques, soils and environmental conditions, as well as, the quality and quantity of the crude oil product in the soil environment. And again, bioremediation may be cost effective but one disadvantage is that it is limited to only those groups of hydrocarbons that are biodegradable.

The success and efficiency in the reduction of crude oil contaminants in soil depends on the capacity to optimize the various environmental conditions in the contaminated site. Bioremediation is the most elegant and pure cleanup solution that results in the transformation of toxic compounds to carbon dioxide and water.

The use of engineering modeling techniques will also help the remediation engineering communities to understand the dynamics structure of microbes and assists in providing the needed insight into details of bioremediation which will be facilitated to make the bioremediation technology safer, reliable and so help to transform bioremediation from a mere practice into a science.

16. Future Perspectives

Except for a few limiting factors, the bioremediation technology has the ability to rejuvenate a contaminated environment effectively. But a combination of the right microorganism and control of the right field parameter will be a step toward achieving a higher and acceptable rate of biodegradation. Though an element of unpredictability still exist in attempt to achieving desired success in bioremediation. A concerted effort by researchers and innovators in this direction and a combination of technologies will give the bioremediation industry a quantum leap.

17. References

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