Universal Journal of Environmental Research and Technology
All Rights Reserved Euresian Publication © 2015 **eISSN 2249 0256**
Available Online at: **www.environmentaljournal.org**
2015 Volume 5, Issue 4: 188-200

Open Access **Research Article**

Kinetic Modelling of Bioremediation of Water Contaminated With Bonny Light Crude Oil Using Biostimulation-Bioaugmentation Agent

Latinwo G. K. and *Agarry S. E.

Biochemical and Chemical Engineering Biotechnology Laboratory, Department of Chemical Engineering, Ladoke Akintola University of Technology, P. M. B. 4000, Ogbomoso, Nigeria.

***Corresponding author: sam_agarry@yahoo.com**

Abstract:

The objective of this study was to investigate the potential use of cassava steep liquor (CSL) alone and in combination with potassium phosphate and ammonium sulphate (inorganic nutrients) as biostimulation-bioaugmentation agent in the bioremediation of crude oil contaminated waters. Bioremediation was carried out for six weeks at ambient temperature in plastic bucket bioreactors containing water artificially contaminated with 10% (v/v) Bonny light crude oil. CSL alone and in combination with potassium phosphate and/or ammonium sulphate was added to each bioreactor. Similar contaminated water without CSL and potassium phosphate/ammonium addition served as control designated as natural bioattenuation. The results revealed that natural bioattenuation, CSL supplementation, CSL + potassium phosphate, CSL + ammonium sulphate, and combined CSL + potassium + ammonium sulphate remediation systems elicited 40%, 58.5%, 67.7%, 64.1% and 77.4% total petroleum hydrocarbon degradation and a corresponding biochemical oxygen demand (BOD) reduction of 52%, 62.7%, 71%, 66.7% and 81.7%, respectively. The microbial load in the contaminated water increased rapidly between days 0 and 7 and decreased between days 14 and 42 in all the remediation systems. A first-order kinetic model fitted well to the biodegradation data and the corresponding half-life time was also estimated. The model revealed that crude oil contaminated water under combined CSL + potassium + ammonium sulphate treatment strategy had higher biodegradation rate constants, k as well as lower half-life times, $t_{1/2}$ than other remediation systems. Thus, the results suggest that CSL and in combination with inorganic nutrient could be an indispensable tool in bioremediation of petroleum hydrocarbons contaminated environment.

Keywords:

Bioremediation; Biodegradation; Cassava steep liquor; Crude oil; Inorganic nutrient; First-order kinetics

1.0 Introduction:

World-wide increase for fossil fuel has led to increase in petroleum exploration, refining and other associated industrial activities in developed and developing countries like Nigeria. Generally, and particularly in the Niger Delta region of Nigeria, these activities have led to the wide scale contamination of its farmland, creeks, swamps, rivers and streams with crude oil and its associated petroleum products (Odokuma and Okpokwasili, 1993; Adebusoye et al., 2010). The problem of crude oil pollution is made worse by sabotage and deliberate vandalization of pipelines in the Niger Delta region of Nigeria. The contamination of these environment constitutes a major public health and socio-economic hazards which often times results in violent protest between the oil companies and its host communities. It is estimated that more than 2 million tons of oil enters marine environments from ships and other sea-based activities annually. Table 1 summarizes total hydrocarbon pollution of marine environments worldwide (GESAMP, 2007; Zahed et al., 2011). The scale of the hazards imposed on the natural environment depends on the surface of the area contaminated by petroleum products, their chemical composition, and the depth at which pollutants occur.

Crude oil is an extremely complex mixture of aliphatic and aromatic hydrocarbons, including volatile components of gasoline, petrol, kerosene, lubricating oil and solid asphaltene residues. Crude oil causes a variety of risks when released into the

environment. It is physically, chemically and biologically harmful to soil and water because of the presence of many toxic compounds, such as polycyclic aromatic hydrocarbons, benzene and its substituted and cyclo alkane rings, in relatively high concentrations. This oil can cause chronic sub-acute toxicological effect (reduced growth and reproduction, poor health, low recruitment rates), which can alter population dynamics and disrupt tropic interactions and the structure of natural communities within ecosystems (Bejarano and Michel, 2010).

The remediation processes leading to the eventual removal of these petroleum hydrocarbons from the environment involve the trio of physical, chemical and biological alternatives (Okoh, 2006). The Physical and chemical methods are the most widely used procedures for clean-up (Ikhajiagbe, and Anoliefo, 2011). However, the physicochemical methods have their limitations (Less and Senior, 1995; Vidali, 2001) and these limitations are: they are expensive to implement at full scale, they are not environmentally friendly, their technologies are complex and they lead to destruction of soil texture and characteristics (Zhang et al., 2009). Furthermore, the physicochemical methods do not always result in complete neutralization of pollutants (Yerushalmi et al., 2003). Due to limitations of the physicochemical technologies stated above, great deals of literature have reported that bioremediation methods are alternatives and or supplements to these methods. This is because of their cost effectiveness, environmental friendliness, simplicity in technology and conservation of soil texture and characteristics (Mandri and Lin, 2007; Adams and Guzmán-Osorio, 2008; Fouépé et al., 2009).

Bioremediation is an ecologically acceptable technology that exploits the diverse degradation abilities of microorganisms to efficiently convert complex chemical components of crude petroleum to harmless products by mineralization. Nevertheless, bioremediation is an emerging technology and still has its limitations of been slow and thus require a longer treatment time. This disadvantage may be due to limitation by physico-chemical as well as biological factors, such as nutrients, pH, temperature, moisture, oxygen, soil properties, and contaminant concentration, number and type or species of microorganisms (Atlas and Bartha, 2006; Kao et al., 2008; Namkoong et al., 2002; Sabate et al., 2004; Walter et al., 2005). Hence, there is the need to increase the rate of degradation or recovery so as to reduce the treatment time, which is regarded as enhanced bioremediation. Enhanced bioremediation encompasses a broad continuum of technologies (Sarkar et al. 2005; Chien et al. 2008) which may involve the addition of electron acceptors or electron donors to stimulate naturally occurring microbial populations (biostimulation) or could be the introduction of specific microorganisms (bioaugmentation) to enhance the biodegradation of the target compound. Microbes and nutrients (that contain nitrogen) have been identified as one of the various factors that may limit the rate of petroleum hydrocarbon degradation. Thus, bioremediation technologies have been developed for soils and water using the addition of nutrients and microbes (Lei et al., 2008; Joo et al., 2008; Greenwood et al., 2008). The addition of microorganisms as pure or mixed culture to degrade petroleum hydrocarbon pollutants (Wolicka et al., 2009) or the use of nutrient supplements such as inorganic fertilizer (Riffaldi et al., 2006; Margesin et al., 2007) and organic fertilizer in terms of animal dungs (Akinde and Obire, 2008; Singh and Fulekar, 2009 ; Agarry et al., 2010) and agricultural crop residues (Abioye et al., 2009; Agarry et al., 2013) to stimulate degraders in polluted matrices has been extensively studied, nevertheless, the search for cost effective and environmentally friendly methods of petroleum hydrocarbon removal from contaminated environment still needs to be further investigated.

While earlier works has focused on the use of isolated and cultured organisms as well as solid-based agricultural wastes residue for enhancing bioremediation, very little study has focused on liquid wastes emanating from agricultural-based industries as potential biostimulation and/or bioaugmentation agents for bioremediation. Adebusoye et al. (2010) and Obidi et al. (2010) have reported the use of cassava steep liquor and fermented cassava steep for the bioremediation of soil contaminated with diesel oil and the bioremediation of crude petroleum polluted stagnant water, respectively. However, both workers did not study the kinetics of bioremediation which is essential to determine the equilibrium constants, speed of reaction and control of the process. The kinetics of bioremediation can be evaluated in two ways (Zahed et al., 2011): (1) the first concerns with the factors influencing the amount of transformed compounds with time and (2) the other approach seeks the types of curves describing the

transformation and determines which of them fits the degradation of the given compounds by the microbial culture (Ron˘cevi´c, et al., 2005).

Therefore, the objective of this study is to determine the biostimulation-bioaugmentation potential of cassava steep liquor in the enhancement of crude oil (petroleum hydrocarbon) biodegradation in water. The degradation kinetics of Bonny light crude oil in water with respect to the liquid wastes and other nutrient supplement were determined, evaluated, compared, and modeled using first-order kinetic model. Cassava steep liquor (CSL) is a waste generated from cassava processing plants which are widely distributed in Nigeria. The indiscriminate discharge of this waste into the environment is a public health concern and thus its use for crude oil bioremediation purposes will help arrest this ecological disaster and further lower the cost of oil spill cleanup since it is readily available nationwide and at no cost.

2.0 Materials and Methods:

2.1 *Collection of Samples:*

Lagoon water samples were aseptically collected from the Lagoon in Lagos, Nigeria. The Bonny light crude oil (API, 31.2 and density, 0.8694 kg/l) was obtained from Nigerian National Petroleum Corporation, Port Harcourt, Nigeria. It was weathered by exposure to the atmospheric condition from 10.00 a.m to 4.00 p.m for two weeks with occasional stirring after which it was stored for further use.

2.2 *Preparation of Cassava Steep Liquor:*

Fresh cassava tubers (Manihot esculenta) were obtained from farm produce market in Ogbomoso, Nigeria. The tubers were peeled and thoroughly washed with tap water and after which they were cut into small pieces. These cut cassava pieces were soaked in tap water inside a clean plastic bucket and left for a period of five days at room temperature. After the five days of steeping, the fermented cassava mash was thoroughly mixed and then strained in a domestic sieved. The samples of steep liquor were collected and were used for the bioremediation study. The microorganisms identified in the cassava steep liquor were *Penicillium* spp, *Aspergillus* spp, *Rhizopus* spp, *Bacillus* spp and *Streptococcus* spp.

2.3 *Bioremediation of Lagoon Water Contaminated With Crude Oil*:

Fifteen plastic buckets with 15 L capacity were used as bioreactors for the study and divided into five groups, each composed of three buckets and labeled A to E. Group A served as the control experiment. Lagoon water (1 L) was poured into the bioreactor in each group and artificially contaminated with 100 ml of Bonny light crude oil. Thereafter, 200 ml of cassava steep liquor (CSL) was added into each of the bioreactor in group B, C, D and E, and to the bioreactors in group C, D and E was added 2% potassium phosphate, 2% ammonium sulphate and a mixture of 1% potassium phosphate and 1% ammonium sulphate, respectively. CSL was not added to the bioreactors in group A which served as control experiment, however, 200 ml of tap water was added. The contents in each bioreactor were thoroughly mixed to allow for aerobic condition. The bioreactors and their contents were incubated for six weeks (42 days) at room temperature (25 ± 3^{o}C). Samples from the experimental and control set-ups were taken aseptically at intervals of 7 days and analyzed for biological oxygen demand (BOD), total heterotrophic bacteria (THB), total hydrocarbon utilizing bacteria (THUB), total heterotrophic fungi (THF) and residual crude oil, respectively.

2.4 *Determination of Residual Crude Oil and Total Petroleum Hydrocarbon:*

The residual crude oil was extracted from both the experimental and control samples using n-hexane: dichloromethane solvent systems (1:1) and was quantified gravimetrically (Yveline et al., 1997) and spectrophotometrically (Adesodun and Mbagwu, 2008). Five milliliter of hexane: dichloromethane solvent system was added to the sample broth solutions and shaken vigorously for 5 min to extract the residual crude oil. The extract was filtered and filtrate centrifuged at 2500 rpm. Two different layers were formed; the upper layer which was organic phase layer contained biodegraded crude oil and lower layer was medium layer. The organic phase layer was collected into a pre-weighed beaker using separating funnel. The process was repeated thrice and the combined organic phase layer was allowed to evaporate to dryness at room temperature and the beaker and its content was weighed after which the residual oil was obtained by difference in mass. Also, the organic phase extract (filtrate) was diluted by taking 1 ml of the extract into 50 ml of hexane. The absorbance of this solution was measured spectrophotometrically at a wavelength of 400 nm HACH DR/2010 Spectrophotometer using n-hexane as blank. The total petroleum hydrocarbon (TPH) in water was estimated with reference to a

standard curve derived from fresh crude oil of different concentration diluted with n-hexane. Percent degradation (D) was calculated using the following formula:

$$\frac{TPH_i - TPH_r}{TPH_i} \times 100 \quad (1)$$

Where TPH_i and TPH_r are the initial and residual TPH concentrations, respectively.

2.5 ***Determination of Biochemical Oxygen Demand:***

The winkler method was used in the estimation of the biochemical oxygen demand (BOD) of the wastewater samples (Woodring and Clifford, 1988).

2.6 ***Determination of Total Heterotrophic Bacteria and Total Heterotrophic Fungi:***

The total heterotrophic bacteria (THB) and total heterotrophic fungi were estimated using nutrient agar (NA) and potato dextrose agar (PDA) plates, respectively. Aliquots (1.0 ml) of the appropriate sample broth were plated on the above media in three replicates, spread aseptically and incubated aerobically with NA plates at 37^{o}C for 1-3 days and PDA plates at 30^{o}C for 3-5 days, respectively. At the end of the incubation period, developed colonies were counted. The relative abundance i.e. the population density estimate of the organisms was obtained by multiplying the plate count per ml for each organism by the dilution factor used (Nwachukwu and Ugoji, 1995).

2.7 ***Determination of Microbial (Total Hydrocarbon-Degrading Bacteria) Count:***

Quantification of the total hydrocarbon-degrading microorganisms (THDB) present in the samples was conducted using the *pour plate* technique. Aliquots (1.0 ml) of the appropriate sample broth were subjected to a serial 10-fold dilution procedure and cultivated in a nutrient agar medium. Three plates were inoculated for each dilution. The plates were incubated at 30 ^{o}C for 48 h and the number of colony forming units (CFU) was counted in each sample. The results were expressed as colony-forming units per ml (CFU/ml). All microbiological counts and experiments were carried out in triplicate.

2.8 ***Kinetic Model Analysis:***

Kinetic analysis is a key factor for understanding biodegradation process, bioremediation speed measurement and development of efficient clean up for a petroleum hydrocarbon contaminated environment. The information on the kinetics of soil bioremediation is of great importance because it characterizes the concentration of the contaminant remaining at any time and permit prediction of the level likely to be present at some future time. Petroleum hydrocarbon biodegradation rates are usually difficult to predict due to the complexity of the environment (Zhu et al., 2001). Nevertheless, biodegradation rate of organic compounds by microorganisms is often described by the equation as follows (Wang et al., 2001):

$$q = \frac{q_m c}{k + c} \quad (2)$$

Where q is biodegradation rate, q_m is maximum specific biodegradation rate, c is the substrate concentration and k is half-saturation constant.

If c « k ; Eq. (2) can be reduced to

$$q = \frac{q_m c}{k} \quad (3)$$

Eq. (3) is a typical first-order model. The use of first-order kinetics in the description of biodegradation rates in environmental fate models is common because mathematically the expression can be easily incorporated into the model (Greene et al., 2000). Assuming $k_1 = (q_m / k)$ and integrating Eq. (3), the following relation of substrate concentration to time can be obtained as given in Eq. (4):

$$\ln c = a + k_1 t \quad (4)$$

2.9 ***Estimation of Biodegradation Half-Life Times:***

The biological half-life is the time taken for a substance to lose half of its amount. Biodegradation half-lives are needed for many applications such as chemical screening (Aronson et al., 2006), environmental fate modeling (Sinkkonan and Paasivirta, 2000) and describing the transformation of pollutants (Dimitrov et al., 2007; Matthies et al., 2008). Biodegradation half-life times ($t_{1/2}$) are calculated by Eq. (5) (Zahed et al., 2011; Agarry et al., 2013):

$$t_{1/2} = \frac{\ln 2}{k} \quad (5)$$

Where k is the biodegradation rate constant (day^{-1}). The half life model is based on the assumption that the biodegradation rate of hydrocarbons positively correlated with the hydrocarbon pool size in soil (Yeung et al., 1997).

3.0 Results and Discussion:

3.1 *Biodegradation of Bonny Light Crude Oil:*

The biodegradation profile of Bonny light crude oil oil in water subjected to natural bioattenuation, cassava steep liquor (CSL) alone (as biostimulation-bioaugmentation agent), CSL + potassium phosphate, CSL + ammonium sulphate and CSL + potassium phosphate + ammonium sulphate treatment, respectively, as a function of remediation time is shown in Fig. 1. It is observed that the percentage reduction in TPH was very rapid within the first 7 days of remediation in the entire treatment systems and this continued slowly up to the sixth week (day 42). At the end of remediation period (day 42), there was 40%, 58.5%, 67.7%, 64.1% and 77.4% TPH degradation in crude oil contaminated water subjected to natural bioattenuation, CSL, CSL + potassium phosphate, CSL + ammonium sulphate, and CSL + potassium phosphate + ammonium sulphate treatment, respectively. This observation revealed that using CSL as biostimulation-bioaugmentation agent during the crude oil biodegradation in water resulted in a more effective bioremediation response than the natural bioattenuation. This observation may be due to the fact that CSL increased the nutrients level as well as the microbial load or density in the lagoon water thus acting as biostimulation and bioaugmentation agent. A similar observation has been reported for the use of CSL in the bioremediation of soil contaminated with diesel oil (Adebusoye et al., 2010) and in the bioremediation of crude petroleum polluted stagnant water using fermented cassava steep (Obidi et al., 2010). Furthermore, the results also revealed that the addition of potassium phosphate, ammonium sulphate and their combinations to CSL elicited a higher biodegradation of crude oil in water than the use of CSL alone. Generally, in this work, the combination of CSL, potassium phosphate and ammonium sulphate treatment strategy showed relatively greater percentage TPH degradation than the use of CSL alone, CSL + potassium phosphate, and CSL + ammonium sulphate, respectively, during the whole period of remediation.

3.2 *Evaluation of Biochemical Oxygen Demand:*

Biochemical oxygen demand is a measure of the amount of oxygen consumed by microorganisms in decomposing organic matter in water bodies. It also measures the chemical oxidation of inorganic matter (i.e., the extraction of oxygen from water via chemical reaction). The BOD profile of the contaminated water at various remediation conditions is shown in Fig. 2. Generally, the BOD value of crude oil contaminated water in all the remediation systems was observed to reduce in the course of bioremediation. The crude oil contaminated under natural bioattenuation (control) showed a decrease in BOD from 120 to 57 mg/l corresponding to 52.5% BOD reduction, indicating that there was an observable level of bioremediation. The reduction in BOD could be attributed to the activities of the indigenous microbes present in the lagoon water. The bioreactor B which contained crude oil contaminated water supplemented with CSL showed a reduction in BOD of from 220 to 82 mg/l corresponding to 62.7% BOD reduction. This indicates that CSL supplementation enhanced the crude oil bioremediation capacity of the indigenous microorganisms. The treatment systems C and D which contained crude oil contaminated water supplemented with CSL + potassium phosphate and CSL + ammonium sulphate showed BOD reductions from 200 to 58 mg/l and from 180 to 60 mg/l which corresponds to 71% and 66.7% BOD reductions respectively. These results highlight the positive influence of supplying inorganic nutrient in combination with the CSL on crude oil contaminated bioremediation. The treatment system E which consists of the combination of CSL + potassium phosphate + ammonium sulphate revealed a reduction of BOD from 180 to 33 mg/l which corresponds to 81.7% BOD reduction. Obahiagbon and Aluyor (2009) and Amenaghawon et al. (2014) have reported enhanced bioremediation levels when crude oil contaminated water were supplemented with nitrates and NPK fertilizer, respectively.

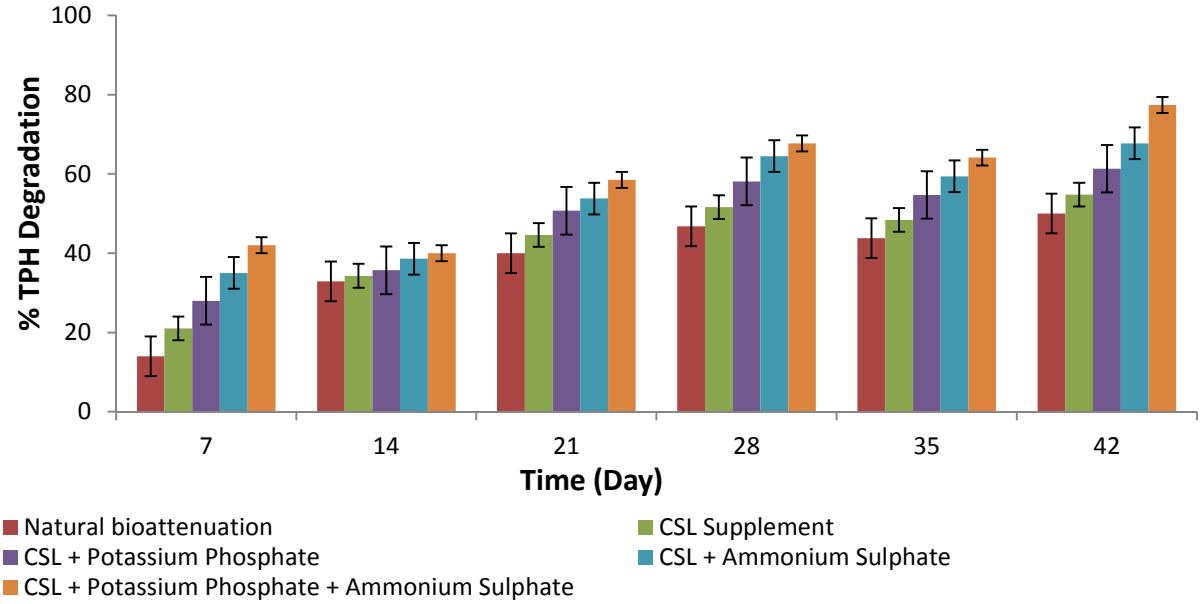


Fig. 1: Time course for the biodegradation of crude oil under natural bioattenuation, CSL supplement, CSL + potassium phosphate, CSL + ammonium sulphate, and combined CSL + potassium phosphate + ammonium sulphate. Bars indicate the average of triplicate samples while the error bars show the standard deviation.

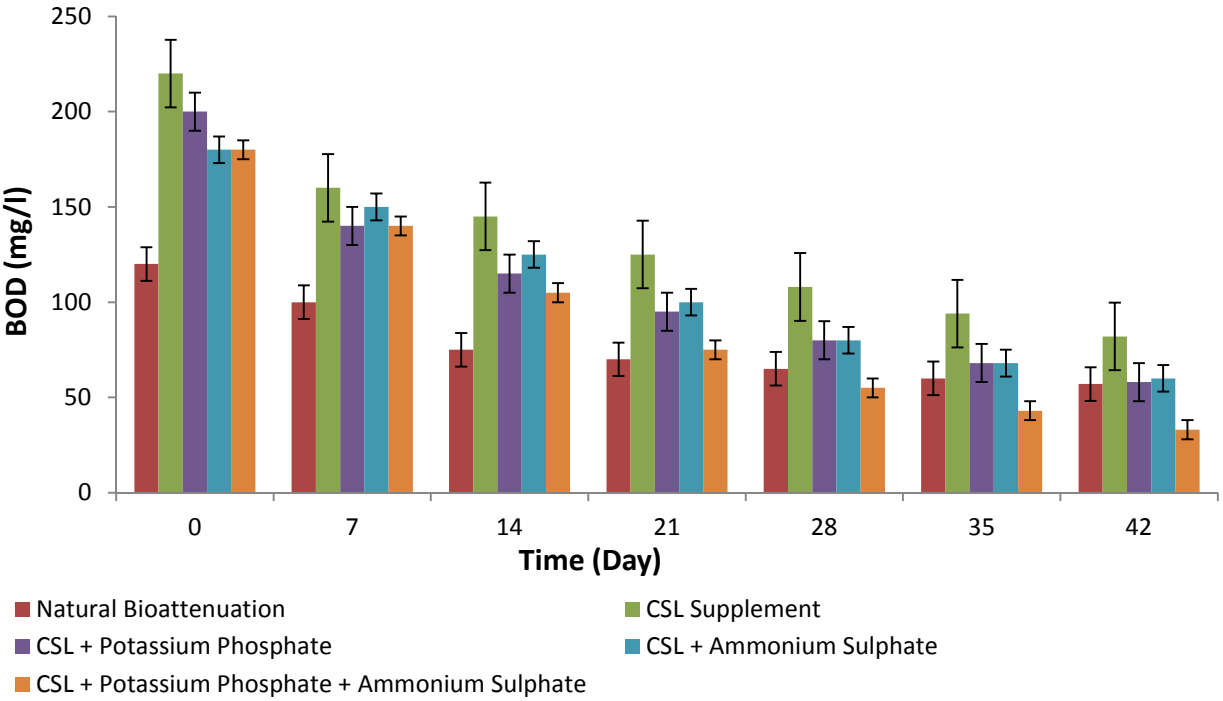
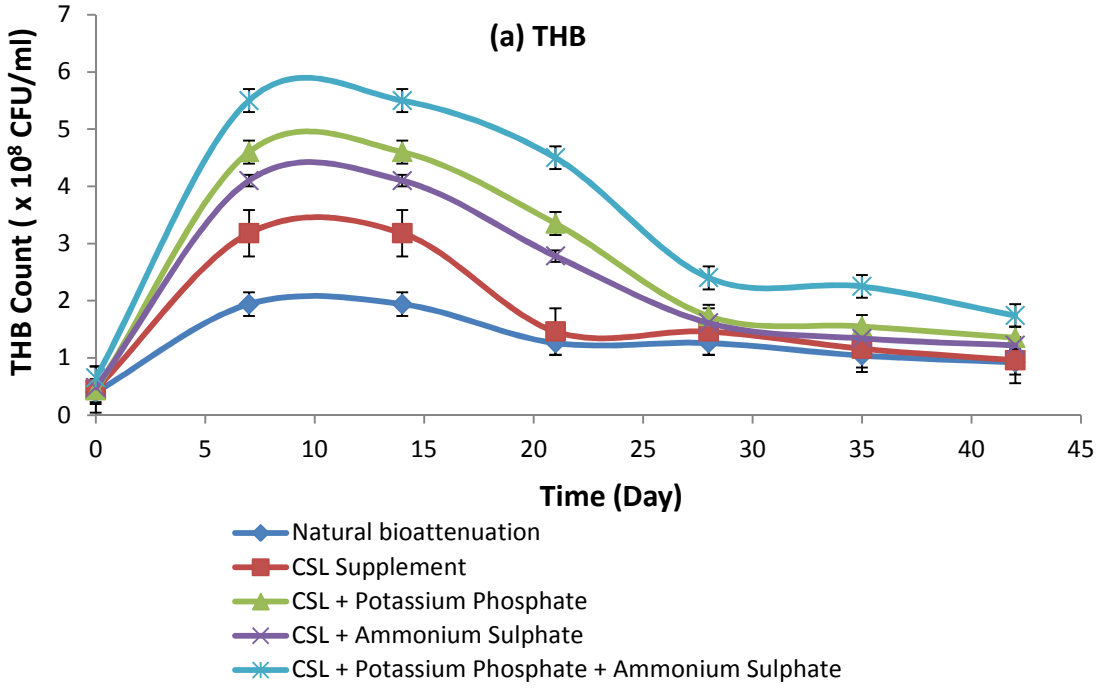
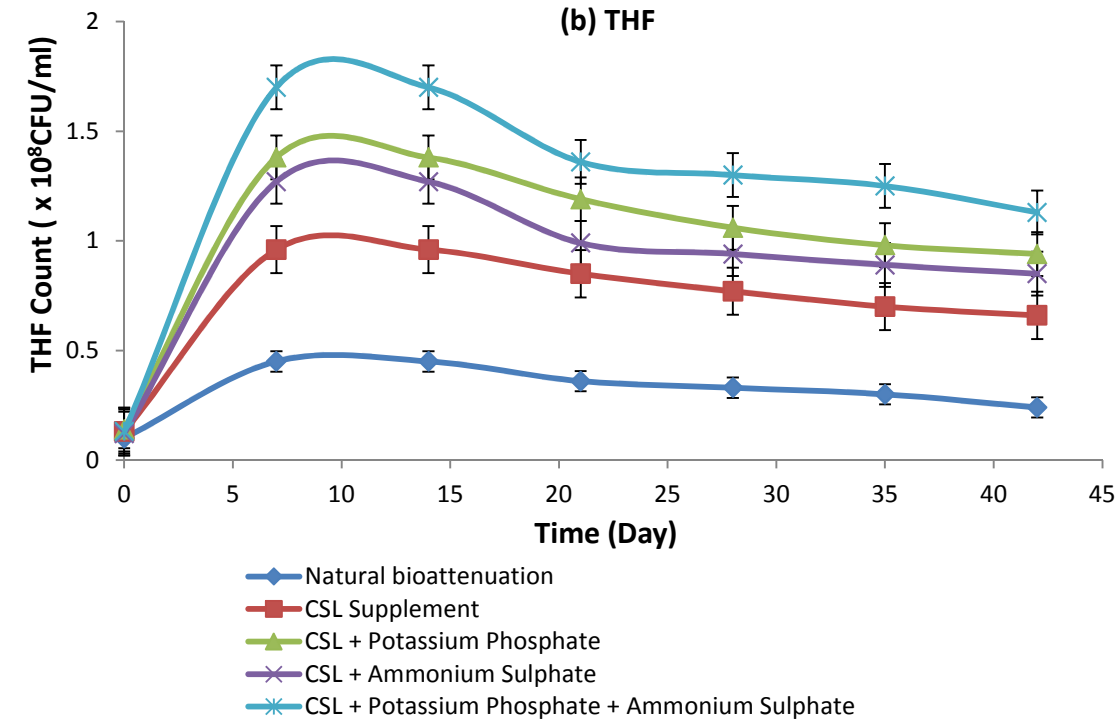


Fig. 2: Time course for BOD reduction during the biodegradation of crude oil under natural bioattenuation, CSL supplement, CSL + potassium phosphate, CSL + ammonium sulphate, and combined CSL + potassium phosphate + ammonium sulphate. Bars indicate the average of triplicate samples while the error bars show the standard deviation.

3.3 *Microbial Growth:*

Fig. 3 (a) – (c) shows the growth profiles of the total heterotrophic bacteria, total heterotrophic fungi and total hydrocarbon utilizing bacteria (THUB) in crude oil contaminated water subjected to natural bioattenuation, CSL alone (as biostimulation-bioaugmentation agent), CSL + potassium phosphate, CSL + ammonium sulphate and CSL + potassium phosphate + ammonium sulphate treatment, respectively. Generally, it is seen that the microbial (THB, THF and THDB) counts increased maximally between day 0 and day 7 in each of the treatment system. As seen in Fig. 3 (a), for natural attenuation, the THB count increased from $0.2 \pm 0.4 \times 10^8$ CFU/ml on day 0 to $0.1 \pm 1.94 \times 10^8$ CFU/ml on day 7 and remained constant until day 14, after which it decreased to $0.3 \pm 0.92 \times 10^8$ CFU/ml on day 42. For CSL alone supplemented crude oil biodegradation, THB count increased from $0.2 \pm 0.45 \times 10^8$ CFU/ml on day 0 to $0.1 \pm 3.18 \times 10^8$ CFU/ml on day 7 and remained constant until day 14, after which it decreased to $0.3 \pm 0.96 \times 10^8$ CFU/ml on day 42. As for the CSL + potassium phosphate supplemented crude oil biodegradation, THB count increased from $0.2 \pm 0.43 \times 10^8$ CFU/ml on day 0 to $0.1 \pm 4.60 \times 10^8$ CFU/ml on day 7 and remained constant until day 14, after which it decreased to $0.3 \pm 1.35 \times 10^8$ CFU/ml on day 42. Furthermore, for the CSL + ammonium sulphate supplemented crude oil biodegradation, THB count increased from $0.2 \pm 0.48 \times 10^8$ CFU/ml on day 0 to $0.1 \pm 4.10 \times 10^8$ CFU/ml on day 7 and remained constant until day 14, after which it decreased to $0.3 \pm 1.22 \times 10^8$ CFU/ml on day 42. While for the CSL + potassium phosphate + ammonium sulphate supplemented crude oil biodegradation, THB count increased from $0.2 \pm 0.65 \times 10^8$ CFU/ml on day 0 to $0.1 \pm 5.50 \times 10^8$ CFU/ml on day 7 and remained constant until day 14, after which it decreased to $0.3 \pm 1.74 \times 10^8$ CFU/ml on day 42. Similar observations were found for THF and THUB, respectively (Fig. 3b and 3c). Fig. 3 (a) – (c) shows that there is no lag phase, while day 0 – 7 indicates an exponential phase (this is the period of maximum growth), day 7 – 14 indicates a stationary phase and day 14 – 42 could be regarded as decelerating or death phase. However, the decrease in microbial growth (i.e. the death phase) observed in all the treatment system after day 14 may probably be due to nutrient depletion and product toxicity. The microbial growth observed in all the treatment system as revealed by the THB and THF growth indicated that both bacteria and fungi were involved in the biodegradation of crude oil in the water. However, THB exhibited higher growth than the THF. The period between day 0 and day 7 where the microbial growth was maximum, the degradation of crude oil was also very rapid while the period between day 14 and day 42 when there was a decrease in growth, so also the crude oil degradation continued slowly as shown in Fig. 1. Nevertheless, the presence of CSL + potassium phosphate + ammonium sulphate in the crude oil contaminated water elicited the greatest microbial growth than other treatment systems.





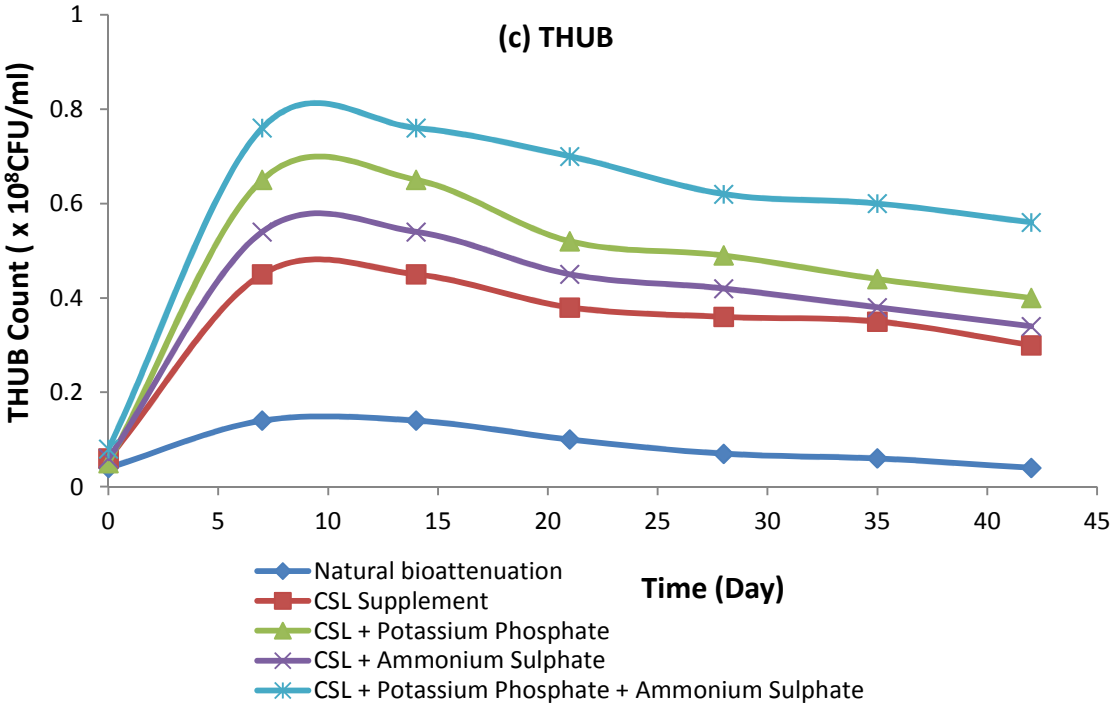


Fig. 3: Time course for the growth of (a) THB, (b) THF, and (c) THUB in crude oil contaminated water under natural bioattenuation, CSL supplement, CSL + potassium phosphate, CSL + ammonium sulphate, and combined CSL + potassium phosphate + ammonium sulphate. Bars indicate the average of triplicate samples while the error bars show the standard deviation.

3.4 ***Evaluation of Biodegradation Kinetics and Half-Life:***

First-order kinetics model equation (Eq. 4) fitted to the biodegradation data (Figure 3 (a) - (c)) was used to determine the rate of crude oil biodegradation in the various remediation treatments.

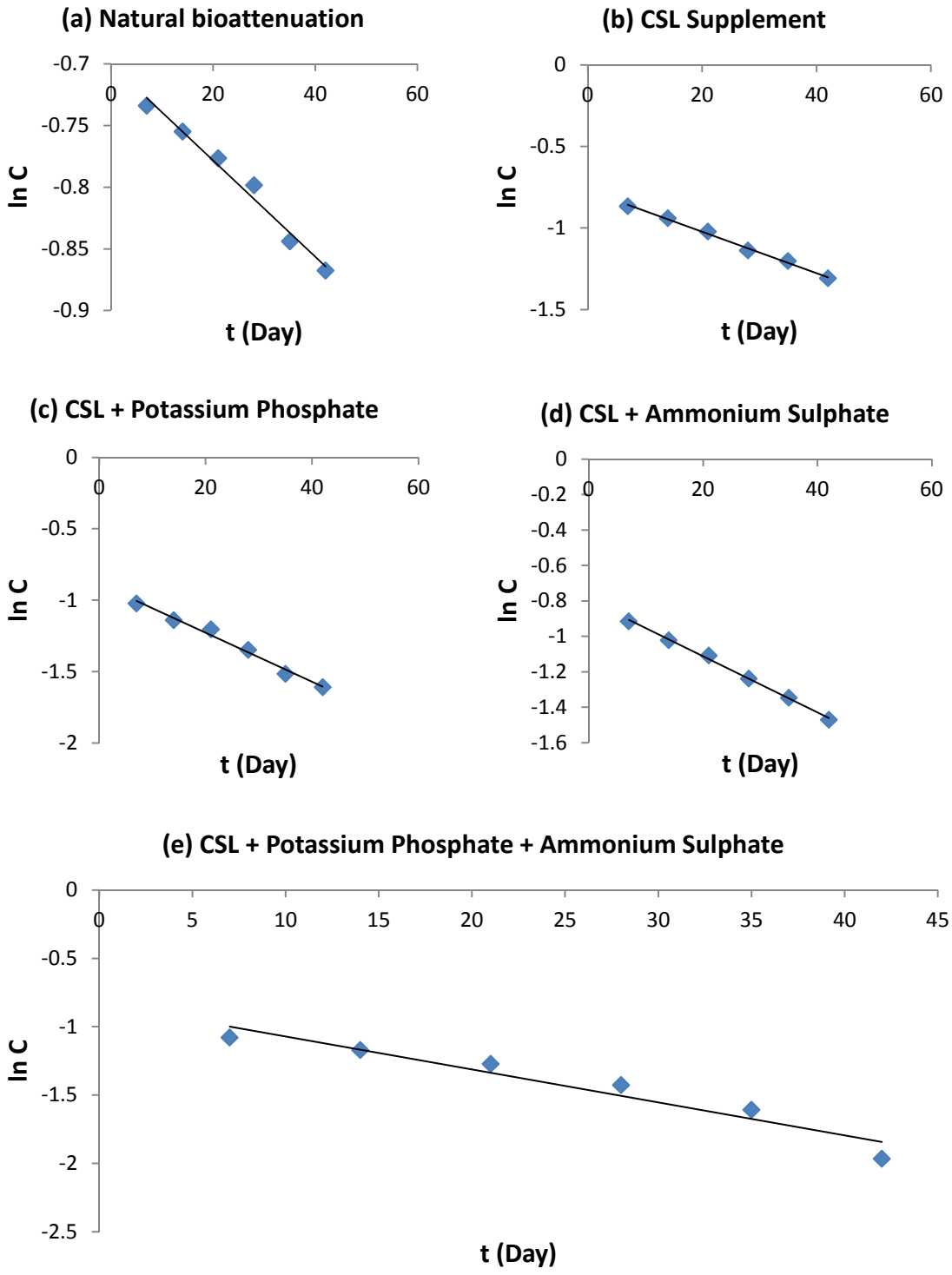


Fig. 4: First-order kinetic model fitted to the biodegradation data of crude oil under (a) natural bioattenuation, (b) CSL supplement, (c) CSL + potassium phosphate, (d) CSL + ammonium sulphate, and (e) CSL + potassium phosphate + ammonium sulphate

The values of the rate constants obtained from fitting of the model are presented in Table 1.

Table 1: First-order kinetic equation with correlation determination (R^2) results of crude oil biodegradation under different treatment systems

Bioremediation System	k (day^{-1})	R^2	Half-life $t_{1/2}$ (days)
Natural Bioattenuation	0.003	0.980	231
Cassava Steep Liquor Supplement	0.012	0.995	57.8
Cassava Steep Liquor + Potassium Phosphate	0.017	0.986	40.8
Cassava Steep Liquor + Ammonium Sulphate	0.015	0.997	46.2
Cassava Steep Liquor + Potassium Phosphate + Ammonium Sulphate	0.024	0.932	28.9

The results in Table 1 as indicated by the high correlation determination (R^2) showed that the biodegradation of crude oil in water fitted well to the first-order kinetic model. The half-life time of crude oil biodegradation was calculated using Eq. (4). The biodegradation rate constants (k) and half-life times ($t_{1/2}$) for the different remediation treatments are presented in Table 1. It is to be noted that the higher is the biodegradation rate constants, the higher or faster is the rate of biodegradation and consequently the lower is the half-life time. Table 1 shows that the biodegradation of crude oil in water under combined CSL + potassium phosphate + ammonium sulphate treatment strategy had a higher k (0.024 day^{-1}) and lower $t_{1/2}$ (28.9 days) than that under CSL + potassium phosphate (k = 0.017 day^{-1} and $t_{1/2}$ = 40.8 days), CSL + ammonium sulphate (k = 0.015 day^{-1} and $t_{1/2}$ = 46.2 days), and natural attenuation (k = 0.003 day^{-1} and $t_{1/2}$ = 231 days), respectively. Therefore, value of the kinetic parameter showed that the degree of effectiveness of these bioremediation strategies in the cleanup of water contaminated with crude oil is in the following order: natural bioattenuation < CSL supplementation < CSL + ammonium sulphate < CSL + potassium phosphate < CSL + potassium phosphate + ammonium sulphate.

4.0 Conclusion:

The present studies confirm that the use of cassava steep liquor as biostimulation-bioaugmentation agent as well as the use of inorganic nutrient that supplies nitrogen, potassium and phosphorus such as potassium phosphate and ammonium sulphate improved the rate of biodegradation in water environment contaminated with crude oil. At the end of 42 days remediation period, the maximum total petroleum hydrocarbon (TPH) removal of 40%, 58.5%, 67.7%, 64.1% and 77.4% and a corresponding BOD reduction of 52%, 62.7%, 71%, 66.7% and 81.7% was obtained for water contaminated with crude oil subjected to natural bioattenuation, CSL supplementation, CSL + potassium phosphate, CSL + ammonium sulphate and combined CSL + potassium + ammonium sulphate treatment or remediation systems, respectively. The biodegradation rate constant obtained from the application of first order kinetics described the rate of crude oil biodegradation with and without CSL/inorganic nutrient (biostimulation/bioaugmentation agent). The rate constant (k) ranges between 0.012 day^{-1} and 0.017 day^{-1} for crude oil contaminated water subjected to CSL and/or CSL combined with potassium phosphate/ammonium sulphate and 0.003 day^{-1} for natural bioattenuation. A half-life time ($t_{1/2}$) of 231 days was observed for biodegradation of crude oil in water under natural bioattenuation. This was reduced to between 28.9 and 58.7 days with the usage of CSL and/or CSL combined with potassium phosphate/ammonium sulphate. The value of the kinetic parameter showed that the degree of effectiveness of these bioremediation strategies in the cleanup of water contaminated with crude oil is in the following order: natural bioattenuation < CSL supplementation < CSL + ammonium sulphate < CSL + potassium phosphate < CSL + potassium phosphate + ammonium sulphate. These findings do not represent a general rule and site-specific studies are needed, the approach used here can be a relevant support tool when designing bioremediation strategies on site.

References:

1) Abioye PO. Abdul Aziz A, and Agamuthu P, (2009). Enhanced biodegradation of used engine oil in soil amended with organic wastes. Water Air Soil Poll., 2009: 173—179.
2) Adams RH, and Guzmán-Osorio FJ, (2008). Evaluation of land farming and chemico-biological stabilization for treatment of heavily contaminated sediments in a tropical environment. Int. J. Environ. Sci. Technol. 5 (2):169-178.
3) Adebusoye SA, Ilori MO, Obayori OS, Oyetibo GO, Akindele KA, and Amund OO, (2010). Efficiency of cassava steep liquor for bioremediation of diesel-oil contaminated tropical agricultural soil. The Environmentalist 30 (1): 24 – 34.
4) Adesodun JK, and Mbagwu JSC, (2008). Biodegradation of waste-lubricating petroleum oil in a tropical alfisol as mediated by animal droppings. Bioresour. Technol. 99 (13): 5659-5665.
5) Agarry SE, and Jimoda LA, (2013). Application of carbon-nitrogen supplementation from plant and animal sources in *in-situ* soil bioremediation of diesel oil: experimental analysis and kinetic modelling. J. Environ. Earth Sci., 3 (7): 51-62.
6) Agarry SE, Owabor CN, and Yusuf RO, (2010). Bioremediation of soil artificially contaminated with petroleum hydrocarbon mixtures: Evaluation of the use of animal manure and chemical fertilizer. Bioremediation J.,14 (4): 189 - 195.
7) Agarry SE, Aremu MO, and Aworanti OA, (2013). Kinetic modelling and half-life study on bioremediation of soil co-contaminated with lubricating motor oil and lead using different bioremediation strategies. Soil and Sediment Contam.- An Int. J. 22 (7): 800 – 816.
8) Akinde SB. and Obire O. (2008). Aerobic heterotrophic bacteria and petroleum-utilizing bacteria from cow dung and poultry manure. World J. Microbiol.Biotechnol.,24: 1999 - 2002.
9) Amenaghawon NA, Osunbor O, and Obahiagbon KO, (2014). Impact of nutrients, aeration and agitation on the bioremediation of crude oil polluted water using mixed microbial culture. Int. J. Scient. Res. Environ. Sci. 2 (2): 43 – 48.
10) Aronson D, Boethling R, Howard P, and Stiteler W, (2006). Estimating biodegradation half-lives for use in chemical screening. Chemosphere 63: 1953 – 1960 (2006).
11) Atlas RM, and Bartha R, (2006). Fate and effects of polluting petroleum in the marine environment. Residue Rev., 49 (1), 49-83.
12) Atlas RM, and Bartha R, (1998). Microbial Ecology: Fundamentals and Applications. Benjamin/Cummings Publishing, Menlo Park, CA.
13) Bejarano AC, and Michel J, (2010). Large-scale risk assessment of polycyclic aromatic hydrocarbons in shoreline sediments from Saudi Arabia: environmental legacy after twelve years of the Gulf war oil spill. Environ. Poll. 158: 1561–1569.
14) Chang LK, Ibrahim D, and Che Omar I, (2011). A laboratory scale bioremediation of Tapis crude oil contaminated soil by bioaugmentation of *Acinetobacter baumannii* T30C. Afri. J. Microbiol. Res. 5(18): 2609-2615.
15) Chien HY, Kao CM, Jou CJ, Yang PY, and Huang CC, (2008). Application of enhanced bioremediation to clean up diesel-oil contaminated soils: Laboratory microcosm study. J. Biotechnol.136: S682.
16) Dimitrov S, Pavlov T, Nedelcheva D, Reuschenbach P, Silvani M., Bias R., Comber M., Low L., Lee C., Parkerton T., and Mekentan O. (2007) A kinetic model for predicting biodegradation. SAR QSAR Environ. Res. 18: 443 – 457.
17) Fouépé TA, Kengni TL, Gurunadha Rao VVS, Ndam NJR, (2009). Transfer of moisture through the unsaturated zone in the tropical forest using the neutron probe. Inter. J. Environ. Sci. Technol. 6 (3): 379-388.
18) GESAMP (2007). Estimates of oil entering the marine environment from sea-based activities (IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection), in: Reports and Studies No. 75, GESAMP, London.
19) Greene EA, Kay JG, Jaber K, Stehmeier LG, and Voordouw G, (2000). Composition of soil microbial communities enriched on a mixture of aromatic hydrocarbons. Appl. Environ. Microbiol. 66: 5282–5289.
20) Greenwood PF, Wibrow S, George SJ, and Tibbett M, (2008). Sequential hydrocarbon biodegradation in a soil from arid coastal Australia treated with oil under laboratory controlled conditions. Organic Geochem. 39: 1336–1346.
21) Ikhajiagbe B, and Anoliefo GO, (2011). Natural attenuation of a 14-month-old waste engine oil polluted soil. J. Soil Sci. Environ. Manage. 2(7): 184-192.

22) Joo H, Ndegwa PM, Shoda M, and Phae C, (2008). Bioremediation of oil-contaminated soil using *Candida catenulata* and food waste. Environ. Poll. 156: 891–896.
23) Kao CM, Chen CY, Chen SC, Chien HY, and Chen YL, (2008). Application of in situ biosparging to remediate a petroleum hydrocarbon spill site: Field and microbial evaluation. Chemosphere, 70: 1492–1499.
24) Lei H, Ting M, Dan L, Feng-lai L, Ru-Lin L, and Guo-qiang L, (2008). Optimization of nutrient component for diesel oil degradation by *Rhodococcus erythropolis.* Marine Poll. Bull. 56: 1714–1718.
25) Less ZM, and Senior E, (1995). Bioremediation. A Practical Solution to Land Pollution: In Clean Technology and the Environment, New York, Chapman and Hall, pp 121-146.
26) Mandri T, and Lin J, (2007). Isolation and characterization of engine oil degrading indigenous microorganisms i n Kwazulu-Natal, South Africa. Afri. J. Biotechnol. 6 (1): 23– 27.
27) Margesin R, Hammerle M, and Tscherko D, (2007). Microbial activity and community composition during bioremediation of diesel-oil-contaminated soil: Effects of hydrocarbon concentration, fertilizers, and incubation time. Microbiol Ecol., 53: 259 – 269.
28) Matthies M, Witt J, and Klasmeier J, (2008). Determination of soil biodegradation half lives from simulation testing under aerobic laboratory conditions: a kinetic model approach. Environ. Poll. 156: 99 – 105.
29) Namkoong W, Hwang EY, Park JS, and Choi JY, (2002). Bioremediation of diesel-contaminated soil with composting. Environ. Pollut. 109: 23–31.
30) Nwachkwu SCU and Ugoji EO, (1995). Impacts of crude petroleum spill on microbial communities of tropical soils. Inter. J. Ecol. Environ. Sci. 21: 169 – 176.
31) Obahiagbon KO, Aluyor EO, (2009). Comparison of the efficiency of sodium nitrate and sodium nitrite as nutrients in the bioremediation of petroleum hydrocarbon polluted water. Scientific Research and Essay, 4(8): 728 - 732
32) Obidi OF, Onuoha AC, and Nwachukwu SC, (2010). Bioremediation of Crude Petroleum Polluted Stagnant Water with Fermented Cassava Steep. Report and Opinion 2(11):81-86]. (ISSN: 1553-9873).
33) Okoh AI, (2006). Biodegradation alternative in the cleanup of petroleum hydrocarbon pollutants. Biotechnol. Mol. Biol. Rev. 1: 38-50.
34) Riffaldi R, Levi-Minzi R, Cardelli R, Palumbo S, and Saviozzi A, (2006). Soil biological activities in monitoring the bioremediation of diesel oil-contaminated soil. Water, Air Soil Poll. 170: 3–15.
35) Roncevic S, Dalmacija B, Ivan cev-Tumbas I, Trickovic J, Petrovic O, Klasnja M, and Agbaba J, (2005). Kinetics of degradation of hydrocarbons in the contaminated soil layer, Arch. Environ. Contam. Toxicol. 49: 27–36.
36) Sabat'e, J, Vinas M, and Solanas AM, (2004). Laboratory scale bioremediation experiments on hydrocarbon-contaminated soils. Int. Biodeter. Biodegrad., 54: 19–25.
37) Sarkar D, Ferguson M, Datta R, and Birnbaum S. (2005). Bioremediation of petroleum hydrocarbons in contaminated soils: Comparison of biosolids addition, carbon supplementation, and monitored natural attenuation. Environ. Poll. 136: 187–195.
38) Singh D, and Fulekar MH, (2009). Bioremediation of benzene, toluene and o-xylene by cow dung microbial consortium, JABs 14: 788–795.
39) Sinkkonen S, and Paasivirta J, (2000). Degradation half-life times of PCDDs, PCDF sand PCBs for environmental fate modeling. Chemosphere 40: 943 – 949.
40) Vidali M, (2001). Bioremediation*:* An overview. J. Appl. Chem. 73(7): 1163-1172.
41) Walter M, Boyd-Wilson KSH, McNaughton D, and Northcott G, (2005). Laboratory trials on the bioremediation of aged pentachlorophenol residues. Int. Biodeter & Biodegr., 55(3): 121-130.
42) Wang, JL., Han LP, Shi HC, and Qian Y, **(**2001). Biodegradation of quinoline by gel immobilized *Burkholderia* sp. Chemosphere 44**:**1041-1046.
43) Wolicka D, Suszek A, Borkowski A, and Bielecka A, (2009)..Application of aerobic microorganisms in bioremediation in situ of soil contaminated by petroleum products. Bioresour. Technol., 100: 3221-3227.
44) Woodring SL, and Clifford DA, (1988). A Comparison of the Graphical and Standard Methods for the
45) Determination of Biochemical Oxygen Demand, Journal (Water Pollution Control Federation), 60(4): 537-542.
46) Yerushalmi, L., Rocheleau, S., Cimpoia, R., Sarrazin, M., Sunahara, G., Peisajovich, A., Leclair, G., Guiot, R.S. (2003). Enhanced bioremediation of petroleum hydrocarbons in contaminated soil, Bioremediation J. 7 (1): 37-51.

47) Yeung PY, Johnson RL, and Xu, JG, (1997). Biodegradation of petroleum hydrocarbons in soil as affected by heating and forced aeration. J. Environ. Quality 26: 1511 - 1576.
48) Yveline LD, Frederick J, Pierre D, Michael G, Jean CB, and Gilbert M, (1997). Hydrocarbon balance of a site which had been highly and chronically contaminated by petroleum Wastes of refinery from 1956 to 1997. Mar. Pollut. Bull. 22: 103 – 109.
49) Zahed MA, Abdul Aziz H, Isa MH, Mohajeri L, Mohajeri S, and Kutty SRM, (2011). Kinetic modeling and half life study on bioremediation of crude oil dispersed by Corexit 9500. J. Hazard Mater. 185: 1027–1031.
50) Zhang H, Dang Z, Zheng LC, Yi XY, (2009). Remediation of soil co-contaminated with pyrene and cadmium by growing maize (Zea mays L.). Inter. J. Environ. Sci. Technol. 6 (1): 69-76.
51) Zhu X, Venosa AD, Suidan MT, and Lee K, (2001). Guidelines for the Bioremediation of Marine Shorelines and Freshwaters Waterlands. US Environmental Protection Agency Office of Research and Development National Risk Management Research Laboratory.