Kinetic Modelling and Half-Life Study on Enhanced Soil Bioremediation of Bonny Light Crude Oil Amended with Crop and Animal-Derived Organic Wastes

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Abstract

In this study, the potential effects of crop and animal-derived organic wastes as nutrient supplements to biostimulate autochthonous microflora for hydrocarbon biodegradation were investigated. Microcosms containing soil were spiked with weathered Bonny light crude oil (WBLCO) (10 % w/w) and amended with various amounts of groundnut shell, beans shell, melon shell, cassava peels, cow dung and pig dung alone or in combinations. The rates of biodegradation of the crude oil were studied for a remediation period of 42 days under laboratory conditions. The results showed that there was a positive relationship between the rate of petroleum hydrocarbons biodegradation and presence of the crop and animal-derived organic wastes alone or in combination in soil microcosms contaminated with crude oil. The WBLCO biodegradation data fitted well to the first-order kinetic model. The model revealed that WBLCO contaminated-soil microcosms amended with crop and animal-derived organic wastes had higher biodegradation rate constants (k) as well as lower half-life times (t½) than soil microcosms amended with NPK fertilizer and unamended soil (natural attenuation) remediation system. The biodegradation rate constant and estimated biostimulation efficiency values showed that among the crop and animal-derived organic wastes used alone and in combinations, pig dung suggest to offer the best biostimulation performance, which was closely followed by the combination of pig dung and cassava peels. The system proposed here is inexpensive, efficient, and environmentally friendly and may thus offer a viable choice for petroleum hydrocarbons-contaminated soil remediation.

Keywords: Bioremediation; Biodegradation; Organic wastes; First-order kinetics; Half-life

Introduction

Crude oil is an extremely complex mixture of aliphatic and aromatic hydrocarbons that causes a variety of risks when released into the environment. It is physically, chemically and biologically harmful to soil because of the presence of many toxic compounds, such as polycyclic aromatic hydrocarbons, benzene and its substituted cycloalkane 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 trophic interactions and the structure of natural communities within ecosystems [1]. The fate and effects of spilled crude oil and its products in soils have already been the subject of several studies [2,3]. Biodegradation of hydrocarbon compounds is one of the most important processes involved in the weathering and eventual removal of oil from the environment, particularly for its non-volatile components. Thus, potentially biodegradation can be used for recovery of sensitive areas such as contaminated shorelines, marshes, and wetlands.

The remediation processes leading to the eventual removal of these petroleum hydrocarbons from the environment involve the trio of physical, chemical and biological alternatives [4]. The physical and chemical methods are the most widely used procedures for clean-up [5]. However, the physicochemical methods have their limitations [6,7] 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 [8]. Furthermore, the physicochemical methods do not always result in complete neutralization of pollutants [9]. 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 [10]. This is because of their cost effectiveness, environmental friendliness, simplicity in technology and conservation of soil texture and characteristics [7,9,11]. Bioremediation is the naturally occurring process by which microorganisms transform environmental pollutants into harmless end-products [10].

Application of bioremediation can be more effective where environmental conditions permit microbial growth and activity; its application often involves the manipulation of environmental parameters to allow microbial growth and degradation to proceed at a faster rate [12]. Enhanced bioremediation encompasses a broad continuum of technologies [13,14] 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. Oil spills result in an imbalance in the carbon–nitrogen ratio at the spill site, because crude oil is essentially a composition of carbon and hydrogen [15]. This cause’s nitrogen and phosphorus limitation in an oil-soaked soil, which retards
the growth of bacteria and the utilization of carbon source(s) [15,16]. Microbes and nutrients 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 coastal areas using the addition of nutrients and microbes [17-19]. Biostimulation can be considered as an appropriate remediation technique for crude oil removal in soil and requires the evaluation of both the intrinsic degradation capacities of the autochthonous microflora and the environmental parameters involved in the kinetics of the in situ process [20].

Kinetics of bioremediation process can be evaluated in two ways: (i) factors influencing the amount of degraded compounds with time and (ii) the types of curves that describe the degradation and determines which of them fits the degradation of the given compounds by the microbial culture [21]. Bioremediation kinetic studies in a natural environment are often empirical, reflecting only the basic knowledge about the microbial density and its activity in the given environment [22]. The prediction of petroleum hydrocarbons biodegradation kinetics is difficult and complicated in most cases due to the fact that different components of crude oil such as aliphatic, aromatic and polynuclear compounds have different degradation rates [23,24]. This is why lighter crude oils (higher API gravity) normally have a faster biodegradation than heavier crude oils. Furthermore, variations in biokinetic constants have been reported for biodegradation of petroleum hydrocarbons carried out in the same conditions [25] and this may be due to differences in experimental techniques or data analysis [26]. Nevertheless, kinetics is still essential to determine the speed of reaction and control of the process in hydrocarbon biodegradation studies; however, there is still a very limited knowledge on the subject of bioremediation kinetics of petroleum hydrocarbons.

Biostimulation in systems controlling different physical and chemical factors has been well documented [26-28]. The addition of inorganic or organic nitrogen-rich nutrients (biostimulation) is an effective approach to enhance the bioremediation process [29]. Walworth et al. [30] examined the effects of nitrogen and phosphorus addition on phenanthrene hydrocarbon biodegradation in four soils and found that phenanthrene biodegradation rates were related to the initial nitrogen and phosphorus concentrations in the soils. Mills and Frankenberger [31] evaluated the effect of phosphorus sources and concentration (100-1000 mg/kg) on diesel fuel degradation, and reported that degradation depended on phosphorus availability. Some sources might supply enough phosphorus to restore the microbial C/P relationship, but become unavailable because of their low solubility. Positive effects of nitrogen amendment using nitrogenous fertilizer on microbial activity and/or petroleum hydrocarbon degradation have been widely demonstrated [32,33]. On the other hand, Seklemova et al. [34] found that the addition of nutrients had no effect on the decontamination of a forest soil contaminated with diesel oil. Nevertheless, addition of nutrients including nitrogen and phosphorus is a standard practice for increasing hydrocarbon degradation [35]. By adding these nutrients, the C/N and C/P ratios of the soil are closer to the bacterial C/N and C/P requirements. However, in developing countries, inorganic chemical fertilizers are costly as [42,43] well as not sufficient for agriculture, let alone for cleaning oil spills.

According to Alexandratos [44] and OECD [45], Western Europe uses more chemical fertilizer than almost any other nation in the world due to heavy subsidies from the government and that they uses livestock manure and crop residues to provide almost half of all external nutrient inputs [46] as well as to improve the soil physicochemical properties. However, to the best of our knowledge there is a dearth of information on the use of protein-based crop residues such as beans shell, melon shell and groundnut shell and carbohydrate based-crop residue such as cassava peels as biostimulating or amendment agents for enhanced bioremediation of petroleum hydrocarbons-contaminated soil. Furthermore, the evaluation and comparison of crop-derived organic wastes (crop residues) and animal-derived organic wastes (animal dung) has not been reported in the literature.

Therefore, the objective of this study is to determine the biostimulation potential of crop-derived organic wastes (beans shell, cassava peels, groundnut shell and melon shell) and animal-derived organic wastes (cow dung and pig dung) in the enhancement of crude oil (petroleum hydrocarbon) biodegradation in soil. The degradation kinetics of Bonny light crude oil in soil with respect to the organic wastes amended soil and unamended soil were determined and modeled using first-order kinetic model.

Methods

Collection of samples

The soil sample used for the study was collected from the top soil surface (0-15 cm) of Ladoke Akintola University of Technology (LAUTECH) agricultural farm land, Ogbomoso, Nigeria. The soil samples were air dried, homogenized, passed through a 2-mm (pore size) sieve and stored in a polyethylene bag and kept in the laboratory prior to use. 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 am to 4.00 pm for two weeks with occasional stirring after which it was stored for further use. NPK fertilizer (20:10:10) was purchased from an agro-chemical store, Ogbomoso, Nigeria. The Cow Dung (CD) was collected from a cow market in Ogbomoso, Nigeria. The Pig Dung (PD) was obtained from the piggy farm of LAUTECH, Ogbomoso, Nigeria. The Groundnut Shells (GS), Melon Shell (MS) and Beans Shell (BS) were obtained from a mill in Saja Area of Ogbomoso, Nigeria. The Cassava Peels (CP) was obtained from a cassava market in Ogbomoso, Nigeria. The entire different amendment agent was each sun dried for two weeks, ground and sieved to obtain uniform size particles. Each amendment agent was stored in a polyethylene bag and kept prior to use. The sanitary measures taken include the methods of collection, storage, handling, and distribution/application of the animal dung in the soil. Hand gloves were used...
during collection, storage and application in soil. Hand shovel was used for collection and package into polyethylene bag. Animal dung packaged in polyethylene bag was covered with aluminum foil. Iron rod was used for mixing of the dung and the contaminated soil.

**Characterization of soil sample and amendment agents**

The soil sample and amendment agents were characterized for total carbon (TOC), total nitrogen (N), total phosphorus, moisture content, and pH according to standard methods. The pH was determined according to the modified method of McLean [47]; total organic carbon was determined by the modified wet combustion method [48] and total nitrogen was determined by the semi-micro-Kjeldhal method [49]. Available phosphorus was determined by Brays No. 1 method [50] and moisture content was determined by the dry weight method. The Total Hydrocarbon Degrading Bacteria (THDB) populations were determined by the vapor phase transfer method [51]. The physicochemical characterized parameters are presented in Table 1.

**Solid-phase experimental design and soil treatment**

Soil samples (1 kg) was put into 20 different plastic bins (microcosm) with a volume of about 3 L and labeled A to T, respectively. The soil in each plastic bins was spiked with 10% (w/w) weathered Bonny light crude oil (WBLCO) and thoroughly mixed together to achieve complete artificial contamination. 10% spiking was adopted in order to achieve severe contamination because above 3% concentration, oil has been reported to be increasingly deleterious to soil biota and crop growth [52]. The soil C:N ratio in each microcosm was adjusted by the addition of 200 g each (as single and/or in combination) of Cow Dung (CD), Pig Dung (PD), Beans Shell (BS), cassava peel (CP), Groundnut Shell (GS), Melon Shell (MS), and NPK fertilizer, respectively, as nitrogen source (Table 2) and thoroughly mixed. It was assumed that the aforementioned quantities of the crop and animal-derived organic wastes and NPK fertilizer applied to the relevant treatment microcosms were well worked to at least 15 cm depth in each plastic bin.

Thus, the equivalents of 5000 kg per hectare of each amendment agents as single or in combinations were applied to each microcosm, respectively. These amounts of each organic waste supplied different amount of kg nitrogen per hectare (Table 2). The moisture content was adjusted to 50% water holding capacity by the addition of sterile distilled water and incubated at room temperature (28 ± 2°C). The content of each bin was filled twice a week for aeration, and the moisture content was maintained at 50% water holding capacity. Plastic bin A with soil and weathered crude oil without amendment agents served as control 1 while Plastic bin T with soil and weathered crude oil amended with NPK fertilizer served as control 2. The experiment was set up in triplicate. In total, 60 microcosms were settled and incubated for six weeks (42 days). Periodic sampling from each plastic bin was carried out at 7-day intervals for 42 days to determine the residual Total Petroleum Hydrocarbon (TPH).

### Total petroleum hydrocarbon determination

The Total Petroleum Hydrocarbon (TPH) content of the soil samples was determined gravimetrically by solvent extraction method of Adesodun and Mbagwu [53]. Soil samples (approximately 10 g)

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**Table 1:** Soil sample and organic wastes physical and chemical analysis.

| Microcosm code | Biotimulation agents and amount used | C:N |
|----------------|-------------------------------------|-----|
| A              | Unamended control soil (natural attenuation) |     |
| B              | Cow dung (CD) | 19:1 |
| C              | Pig dung (PD) | 31:1 |
| D              | Cassava peels (CP) | 34:1 |
| E              | Melon shell (MS) | 69:1 |
| F              | Beans shell (BS) | 42:1 |
| G              | Groundnut shell (GS) | 37:1 |
| H              | 100 g Cow dung (CD)+100 g Groundnut shell (GS) | 25:1 |
| I              | 100 g Cow dung (CD)+100 g Melon shell (MS) | 30:1 |
| J              | 100 g Cow dung (CD)+100 g Cassava peels (CP) | 24:5:1 |
| K              | 100 g Pig dung (PD)+100 g Beans shell (BS) | 19:1 |
| L              | 100 g Pig dung (PD)+100 g Groundnut shell (GS) | 18:7:1 |
| M              | 100 g Pig dung (PD)+100 g Cassava peels (CP) | 18:1 |
| N              | 100 g Groundnut shell (GS)+100 g Cassava peels (CP) | 35:1 |
| O              | 100 g Beans shell (BS)+100 g Cassava peels (CP) | 38:1 |
| P              | 100 g Cow dung (CD)+100 g Pig dung (PD) | 15:1 |
| Q              | 100 g Groundnut shell (GS)+100 g Beans shell (BS) | 39:1 |
| R              | 100 g Groundnut shell (GS)+100 g Melon shell (MS) | 48:1 |
| S              | 100 g Beans shell (BS)+100 g Melon shell (MS) | 52:1 |
| T              | 1.75 g NPK fertilizer | 46:1 |

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**Table 2:** Types of organic wastes and their combinations in different microcosms.
was taken from each microcosm and put into a 50-ml flask and 20 ml of n-hexane was added. The mixture was shaken vigorously on a magnetic stirrer for 30 min to allow the hexane extract the oil from the soil sample. The solution was then filtered using a Whatman filter paper and the liquid phase extract (filtrate) diluted by taking 1ml 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 in soil was estimated with reference to a standard curve derived from fresh crude oil of different concentration diluted with n-hexane. Percentage degradation (D) was calculated using the following formula:

\[ D = \frac{TPH_i - TPH_f}{TPH_i} \times 100 \]  

where \( TPH_i \) and \( TPH_f \) are the initial and residual TPH concentrations, respectively.

**Dehydrogenase activity determination**

Soil microbial activity was estimated by the dehydrogenase assay. Dehydrogenase activity was determined by monitoring the rates of reduction of 2, 3, 5-triphenyltetrazolium chloride to triphenylformazan as described by Alef [54], calculated as l g of formazan per gram of soil after one day (24 h), and expressed as relative activity (%) in relation to the control activity (100%).

**Data analysis**

The data were subjected to one-way analysis of variance (ANOVA) at 5% probability. Mean of the different treatments were tested for level of significant differences at p<0.05 by Tukey (Honestly Significant Difference) test. The data analysis was performed using statistical package for social sciences, version 16.0 (SPSS Inc., Chicago, IL, USA).

**Bioremediation kinetics**

Kinetic analysis is a key factor for understanding biodegradation process, bioremediation speed measurement and development of efficient clean up for a crude oil 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. Biodegradability of crude oil is usually explained by first order kinetics [22,23,37] and this is given as in Eq. (2):

\[ C_t = C_0e^{-kt} \]  

where \( C_0 \) is the initial TPH content in soil (mg/kg), \( C_t \) is the residual TPH content in soil at time \( t \) (mg/kg), \( k \) is the biodegradation rate constant (day\(^{-1}\)) and \( t \) is time (day). Plotting the logarithm of TPH concentration versus time presents appropriate information about the biodegradation rate.

**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 [55], environmental fate modeling [56] and describing the transformation of pollutants [57,58]. Biodegradation half life times (\( t_{1/2} \)) are calculated by Eq. (3) [24,36,57]:

\[ t_{1/2} = \frac{\ln 2}{k} \]  

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 [59].

**Results and Discussion**

**Removal of total petroleum hydrocarbons and microbial growth**

The level of biodegradation of WBLCO in soil amended with crop residues and animal dung wastes (alone and in combination) are shown in Figures 1-4, respectively. Figure 1 shows the degradation profile of crude oil and growth profile of THDB in soil amended alone with CD, PD, CP, MS, BS, GS, and NPK fertilizer, respectively. There was a rapid TPH reduction (Figure 1a) with corresponding rapid growth of the THDB (Figure 1c) due to increased microbial activity (Figure 1b) within the first 21 days of the study in all the amended soil (biostimulation) compared to that of unamended soil (natural attenuation). At the end of the 21 days, there was 90.2%, 89.3%, 88.5%, 88%, 87.9%, 86.9%, and 85% TPH reduction in soil amended with PD, CD, GS, MS, BS, CP, and NPK fertilizer, respectively, while 55.6% TPH reduction was observed in unamended soil (natural attenuation). However, at the end of remediation period (day 42), there was 96.6%, 94.9%, 93.8%, 93.5%, 93.1%, 92.7%, and 90.8% TPH reduction in soil.
During this period, microbial activity increased by 3.73, 3.22, 2.93, 2.83, 2.49, 2.15, and 1.7 fold (Figure 1b) with a corresponding increase in THDB growth from 2.1 to 13.4 × 10^6 cfu/g, 1.85 to 10 × 10^6 cfu/g, 1.92 to 10.95 × 10^6, 1.5 to 9.2 × 10^6 cfu/g, 1.4 to 8.1 × 10^6 cfu/g, 1.24 to 7 × 10^6 cfu/g, and 0.715 to 6.08 × 10^6 cfu/g in soil amended with PD, CP, CD, GS, MS, BS, and NPK fertilizer, respectively. At the end of day 42, 67.1% TPH reduction with a THDB growth increase from 1.84 to 12.9 × 10^6 cfu/g, 1.55 to 11.8 × 10^6 cfu/g, 1.47 to 11.21 × 10^6 cfu/g, 1.86 to 10.55 × 10^6 cfu/g, 1.74 to 10.25 × 10^6 cfu/g and 1.88 to 9.55 × 10^6 cfu/g (Figure 2a) was observed at the end of day 42 remediation period for soil amended with PD+CP, CD+GS, CD+MS, CD+CP, PD+BS and PD+GS, respectively (Figure 2a). Furthermore, increase in microbial activity by 2.86, 2.97, 3.12, 3.32, 3.44 and 3.57 fold (Figure 2b) with a corresponding increase in THDB growth from 1.84 to 12.9 × 10^6 cfu/g, 1.55 to 11.8 × 10^6 cfu/g, 1.47 to 11.21 × 10^6 cfu/g, 1.86 to 10.55 × 10^6 cfu/g, 1.74 to 10.25 × 10^6 cfu/g and 1.88 to 9.55 × 10^6 cfu/g (Figure 2c) was observed at the end of day 42 remediation period for soil amended with PD+CP, CD+GS, CD+MS, CD+CP, PD+BS and PD+GS, respectively.

The degree of biodegradation of WBLCO in soil amended with two level combinations of crop residues and/or animal dung wastes (PD+CP, CD+GS, CD+MS, CD+CP, PD+BS and PD+GS) are shown in Figure 2-4, respectively. A rapid TPH reduction within the first 21 days for each combination treatment. During this period, there was 91.96%, 90.86%, 90.92%, 89.45%, 92.58% and 86.61% TPH reduction in soil amended with PD+CP, CD+GS, CD+MS, CD+CP, PD+BS and PD+GS, respectively (Figure 2a). Furthermore, increase in microbial activity by 2.86, 2.97, 3.12, 3.32, 3.44 and 3.57 fold (Figure 2b) with a corresponding increase in THDB growth from 1.84 to 12.9 × 10^6 cfu/g, 1.55 to 11.8 × 10^6 cfu/g, 1.47 to 11.21 × 10^6 cfu/g, 1.86 to 10.55 × 10^6 cfu/g, 1.74 to 10.25 × 10^6 cfu/g and 1.88 to 9.55 × 10^6 cfu/g (Figure 2c) was observed at the end of day 42 remediation period for soil amended with PD+CP, CD+GS, CD+MS, CD+CP, PD+BS and PD+GS, respectively.

The degree of biodegradation of WBLCO in soil amended with two level combinations of crop residues (GS+MS, GS+BS, GS+CP, BS+MS, BS+CP and unamended soil microcosm (natural attenuation)) were observed. However, at the end of day 42 remediation trial, 96.18%, 95.42%, (95.19%), 94.44%, 94.26% and 93.59% TPH reductions were observed for WBLCO contaminated soil amended with NPK, GS+MS, GS+BS, GS+CP, BS+MS, BS+CP and unamended soil microcosm (natural attenuation). Bars indicate the average of triplicate samples while the error bars show the standard deviation.

Figure 2: (a) Degradation profile, (b) Microbial activity and (c) Growth profile of THDB for the biodegradation of WBLCO in soil microcosms amended with NPK, PD+GS, PD+BS, CD+CP, CD+MS, CD+GS, PD+CP and unamended soil microcosm (natural attenuation). Bars indicate the average of triplicate samples while the error bars show the standard deviation.

Figure 3: (a) Degradation profile, (b) Microbial activity and (c) Growth profile of THDB for the biodegradation of WBLCO in soil microcosms amended with NPK, GS+MS, GS+BS, GS+CP, BS+MS, BS+CP and unamended soil microcosm (natural attenuation). Bars indicate the average of triplicate samples while the error bars show the standard deviation.
BS+CP (92.13%) GS+CP (92.13%) and BS+CP (92.13%) as compared to 90.8% and 67.1% TPH reduction in NPK fertilizer amended soil and unamended control soil (natural attenuation), respectively (Figure 3a). Also, at the end of day 42 remediation period there was an increase in the microbial activity by 2.93, 3.00, 3.30, 3.40 and 3.51 fold with a corresponding increase in the growth of THDB from 1.6 to 12.35 × 10^6 cfu/g (Figure 4b and 4c).

Figure 4a shows the level of WBLCO biodegradation in soil amended with NPK, CD+PD and unamended soil microcosm (natural attenuation). Bars indicate the average of triplicate samples while the error bars show the standard deviation.

BS+CP (92.13%) GS+CP (92.13%) and BS+CP (92.13%) as compared to 90.8% and 67.1% TPH reduction in NPK fertilizer amended soil and unamended control soil (natural attenuation), respectively (Figure 3a). Also, at the end of day 42 remediation period there was an increase in the microbial activity by 2.93, 3.00, 3.30, 3.40 and 3.51 fold with a corresponding increase in the growth of THDB from 1.6 to 12.35 × 10^6 cfu/g, 1.7 to 11.88 × 10^6 cfu/g, 1.35 to 11.48 × 10^6 cfu/g, 1.66 to 10.86 × 10^6 cfu/g and 1.5 to 6.6 × 10^6 cfu/g in the WBLCO soil microcosms amended with GS+MS, BS+MS, BS+CP, GS+CP and BS+CP, respectively (Figure 3b and 3c). However, an increase in the microbial activity by 1.67 and 1.70 fold with a corresponding increase in THDB growth from 0.715 to 6.08 × 10^6 cfu/g and 0.30 to 4.67 × 10^6 cfu/g was achieved in soil microcosms amended with NPK fertilizer and unamended soil, respectively.

Figure 4a shows the level of WBLCO biodegradation in soil amended with two level combinations of animal dung waste (CD and PD). It was observed that within the first 21 days of remediation trial there was a fast reduction in TPH and this gradually reduced till the end of remediation period. At the end of day 42 remediation period, 94.44% TPH reduction was achieved as compared to 90.8% and 67.1% TPH reduction observed in the NPK fertilizer amended and unamended control soil (natural attenuation). During this period there was an increase in microbial activity by 3.34 fold with a corresponding increase in THDB growth from 1.89 to 10.56 × 10^6 cfu/g (Figure 4b and 4c).

Therefore, the results depicted in Figures 1-4 revealed that the highest rate of WBLCO degradation was achieved in the first three weeks of remediation. During this period, more than 50% of the TPH was degraded, with a small and continual decrease in degradation until the end of remediation period (six weeks). Biodegradation of WBLCO was high (92% to 96.6%) in all the soil amended with organic wastes as compared to the NPK fertilizer amended (90.8%) and unamended soil (67.1%). Also, the THDB growth and microbial activity in all the amended (crop and animal-derived organic wastes) soil microcosms (biostimulation) was higher than that of the unamended control soil (natural attenuation). This showed that the soil microcosms amended with the crop and animal-derived organic wastes (alone or in combination) enhanced the microbial growth rate which accounted for the higher bacterial counts and microbial activity observed in all the amended soil microcosms than the unamended soil microcosm (natural attenuation). The higher bacterial count in amended soil microcosms (biostimulation) may be due to high nutrient level (in the form of N, P and K) provided by the added crop residues and animal dung organic wastes which stimulated increase in the intrinsic bacterial population and activity thus leading to high energy (carbon) demand by the oil-degrading microbes. This has resulted in the higher reduction of total petroleum hydrocarbon (TPH) (i.e., higher WBLCO degradation) in the amended soil microcosms. Similar observations have been reported for the use of the mixture of rice straw and pig dung [60], mixture of cow dung, pig dung and poultry dung [61], cocoa pod husk and plantain peels [62], and mixture of cow dung and poultry litters [63] as amendment or biostimulation agents in the biodegradation of petroleum hydrocarbons in soil.

Evaluation of biodegradation kinetics and half-life

First-order kinetics model equation (Eq. 2) fitted to the biodegradation data was used to determine the rate of biodegradation of WBLCO in the various remediation treatments which is illustrated in Figures 5-7. The half-life times of WBLCO biodegradation was calculated using Eq. (3). The biodegradation rate constants ($k_1$, $k_2$, $k_3$, $k_4$) and half-life times ($t_{1/2}$) for the different remediation treatments are presented in Table 3. 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 times. It could be seen from Table 3 that among the WBLCO soil microcosms amended alone with animal organic waste (CD and PD), the soil microcosm amended with PD had a higher k (0.0498 day⁻¹) and lower $t_{1/2}$ (13.9 days) than that with amended with CD (k=0.0266 day⁻¹ and $t_{1/2}$=26.1 days). Among the WBLCO soil microcosms amended alone with crop residue organic wastes (CP, GS, MS, and BS), soil microcosm amended with CP had a higher k (0.0288 day⁻¹) and lower $t_{1/2}$ (24.1 days) than others. This was closely followed by soil microcosm amended with GS (k=0.0260 day⁻¹ and $t_{1/2}$=26.7 days), MS (k=0.0257 day⁻¹ and $t_{1/2}$=27.0 days), and BS (k=0.0251 day⁻¹ and $t_{1/2}$=27.6 days), respectively.

More also, for soil microcosms amended with two level combinations of animal and crop residue organic wastes, the soil microcosm amended with PD+CP had a higher k (0.0394 day⁻¹) and lower $t_{1/2}$ (17.6 days) than other amended soil microcosms. However, this was closely followed by soil microcosm amended with CD+GS...
Furthermore, among the WBLCO soil contaminated microcosms amended with two level combinations of crop residue organic wastes, a higher biodegradation rate constant (with $k=0.0351 \text{ day}^{-1}$) and lower half-life times (19.7 days) was attained in the soil microcosm amended with GS+MS than that obtained in other soil microcosms. This was relatively followed by soil microcosm amended with GS+BS ($k=0.0299 \text{ day}^{-1}$; $t_{1/2}=23.2$ days), GS+CP ($k=0.0291 \text{ day}^{-1}$; $t_{1/2}=24.5$ days), BS+MS ($k=0.0270 \text{ day}^{-1}$; $t_{1/2}=25.7$ days), and BS+CP ($k=0.0247 \text{ day}^{-1}$; $t_{1/2}=28.1$ days), respectively. Nevertheless, WBLCO soil contaminated microcosms amended with two level combination of animal organic wastes (CD+PD), the biodegradation rate constant ($k$) and half-life times ($t_{1/2}$) was found to be 0.0283 day$^{-1}$ and 24.5 days, respectively. The biodegradation rate constant ($k$) and half-life time ($t_{1/2}$) for the WBLCO soil contaminated microcosm amended with NPK fertilizer was found to be 0.0228 day$^{-1}$ and 30.4 days, respectively, and for soil microcosm not amended with either of the animal organic wastes or crop residue organic wastes was obtained to be 0.0144 day$^{-1}$ and 48.1 days, respectively.

Thus, the biodegradation rate constants obtained for the different WBLCO soil contaminated microcosms amended with the animal and crop residue organic wastes either alone or in combinations were higher with lower half-life times than that of soil microcosm amended with NPK fertilizer and the unamended (natural attenuation). These observations indicate that the addition of various crop residues and animal dung (organic wastes) alone or in combinations enhanced TPH.
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Effectiveness of biostimulation supplements

A one-way ANOVA analysis was conducted to compare the bioremediation efficiency of the biostimulation or amendment agents and the result is presented in Table 4. The result suggests that the biostimulation or amendment agents had a statistically significant effect on the biodegradation of WBLCO in soil at the 5% probability level (p < 0.05). The effectiveness of each biostimulation agents was therefore tested. Through evaluation of unamended soil microcosm (natural attenuation) and amended soil microcosm (biostimulation), biostimulant efficiency (B.E) was calculated at the end of the 42-day remediation period using Eq. (4) [23]:

\[
%B.E = \frac{\%TPH_{(S)} - \%TPH_{(U)}}{\%TPH_{(S)}} \times 100
\]

where, \(\%TPH_{(S)}\) is the removal of crude oil in the amended soil, and \(\%TPH_{(U)}\) the removal of crude oil in the unamended soil. The results of B. E are illustrated in Table 5.

As presented in Table 5, each of the biostimulant efficiency (% B.E) lies between 26.1 and 30.5%. The results in Table 5 generally showed that the biostimulation efficiency of the crop and animal-derived organic wastes are marginally and relatively close. Thus, post hoc comparisons using Tukey’s (HSD) test at 5% probability level were carried out to actually determine the significant difference in biodegradation efficiency between any of the biostimulation or amendment agents. The difference in TPH concentration mean between pairs of biostimulation treatments were greater than the HSD value, hence, the grouping of TPH mean using the Tukey’s test for the different treatments as presented in Table 6 shows a much significant differences for the bioremediation processes. All the treatments show a significantly different biodegradation rate among them. That is, the Tukey’s test revealed that there are significant differences in the biostimulation efficiency between the control (natural attenuation) and NPK fertilizer as well as the crop and animal-derived organic wastes (alone or in combination). It also indicates that there are significant differences in the biostimulation efficiency between the NPK fertilizer and the crop and animal-derived organic wastes (alone or in combination) while it further showed that there are significant differences in the biostimulation efficiency of all the crop and animal-derived organic wastes (alone or in combination).

### Table 3: The biodegradation rate constants (k) and half-life (\( t_{1/2} \)) time of the various treatments.

| Biostimulation treatment | k (day\(^{-1}\)) | \( R^2 \) | \( t_{1/2} \) (days) |
|--------------------------|----------------|--------|-------------------|
| Animal Waste:            |                |        |                   |
| Pig dung                 | 0.0498         | 0.9572 | 13.9              |
| Cow dung                 | 0.0296         | 0.9525 | 26.1              |
| Crop Residue Waste:      |                |        |                   |
| Cassava peel             | 0.0288         | 0.9519 | 24.1              |
| Groundnut shell          | 0.0260         | 0.9693 | 26.7              |
| Melon shell              | 0.0257         | 0.9670 | 27.0              |
| Beans shell              | 0.0251         | 0.9886 | 27.6              |
| Animal and Crop Residue Wastes Combination: | | | |
| Pig dung+Cassava peel    | 0.0394         | 0.9306 | 17.6              |
| Cow dung+Groundnut shell | 0.0328         | 0.9678 | 21.1              |
| Cow dung+Melon shell     | 0.0296         | 0.9567 | 23.4              |
| Cow dung+Cassava peel    | 0.0289         | 0.9588 | 24.0              |
| Pig dung+Beans shell     | 0.0277         | 0.9518 | 25.0              |
| Pig dung+Groundnut shell | 0.0262         | 0.9662 | 26.5              |
| Crop Residue Wastes Combination: | | | |
| Melon shell+Groundnut shell | 0.0351     | 0.9826 | 19.7              |
| Groundnut shell+Beans shell | 0.0299     | 0.9510 | 23.2              |
| Groundnut shell+Cassava peel | 0.0291     | 0.9651 | 23.8              |
| Melon shell+Beans shell | 0.0270         | 0.9730 | 25.7              |
| Beans shell+cassava peel | 0.0247         | 0.9673 | 26.1              |
| Animal Wastes Combination: | 0.0283         | 0.9653 | 24.5              |
| Inorganic Chemical Fertilizer: | | | |
| NPK Fertilizer (20:10:10) | 0.0228 | 0.9620 | 30.4              |
| Unamended Soil (Control 1) : | | | |
| Natural attenuation | 0.0144         | 0.9993 | 48.1              |

### Table 4: Analysis of variance (ANOVA) for the different treatments.

| Source | Sum of squares | Degree of freedom | Mean of squares | F-value | P-value |
|--------|----------------|-------------------|----------------|---------|---------|
| Treatment | 36813848 | 19 | 1937571 | 10453692 | 0.0000 |
| Error | 14.8284 | 80 | 0.165348 | |
| Total | 36813863 | 99 | |

### Table 5: Percentage degradation of crude oil and biostimulant efficiency at the end of six weeks.
Citation: Agarry SE, Aremu MO, Aworanti OA (2013) Kinetic Modelling and Half-Life Study on Enhanced Soil Bioremediation of Bonny Light Crude Oil Amended with Crop and Animal-Derived Organic Wastes. J Pet Environ Biotechnol 4: 137. doi:10.4172/2157-7463.1000137

Therefore, between the two different animal dung (CD and PD), PD suggests to be relatively more effective with higher B.E (30.5%) than CD (28.4%). Also, between the four different crop residues (GS, MS, BS, and CP), CP suggests to be more effective with a higher B.E (29.2%) than others. However, it is closely followed by GS (28.2%), MS (27.9%), and BS (27.6%). Furthermore, among the two level combinations of animal and crop residue organic wastes, PD+CP suggest to be more effective with higher B.E (30.2%) than other forms of combination. This is closely and relatively followed by CD+GS (29.7%), CD+MS (29.5%), CD+CP (28.9%), PD+BS (28.8%), and PD+GS (28.3%), respectively. For the two level combinations of crop residue organic wastes, GS+MS with higher B.E (29.8%) suggest to be relatively more effective than other combinations. However, this is marginally followed by GS+BS (29.6%), GS+CP (29.4%), BS+MS (28.5%), and BS+CP (27.1%), respectively. Mean while, the B.E for the two level combination of only animal organic wastes (CD+PD) is 28.9% which is lower than that of PD but relatively higher than that of CD. The results in this study suggest that the crop residue and animal-derived organic wastes used as biostimulation agents (alone or in combination) has relative higher biostimulation efficiency in the biodegradation of petroleum hydrocarbons than inorganic (chemical) fertilizer. Moreover, the relative higher efficiency of pig dung, poultry dung and goat dung (animal manure) as biostimulating agents over chemical fertilizer (inorganic nutrient) in the biodegradation of petroleum hydrocarbons in soil has earlier been reported by Agarry et al. [40]. However, this is subject to the amount of animal/plant organic waste and inorganic NPK fertilizer that is being used in the remediation process.

Generally, the difference in the effectiveness (% B.E) of the various crop and animal-derived organic wastes used alone and/or in combination as biostimulant in the enhancement of crude oil biodegradation may be attributed to their specific composition, content and the fiber structure. The cellulose, hemi-cellulose, lignin and nitrogen ratio in the different crop residues as well as in the animal dung wastes may be important factors which regulate microorganism growth and activity [66]. Furthermore, Molina-Barahona et al. [20] have reported that the addition of corn and sugarcane bagasse (crop residue) as a biostimulant in a system to remove diesel oil from contaminated soils affected the contaminant degradation efficiency due to the composition of the bulking agent (hemi-cellulose, cellulose, lignin and nitrogen ratio). A similar observation was also reported for the use of sawdust in the removal of oil and grease in a contaminated soil [25].

The addition of bulking agents to soil has been reported to increase oxygen diffusion and mineral nutrient availability as well as carbon source quality and mechanical support surface for bacterial adsorption, and improves soil physicochemical characteristics as to speed up microbial adaptation and selection [20,25,26,67]. Thus, in our system, the results suggested that both crop residues (plant organic wastes) and animal dung wastes (animal organic wastes) alone and/or in combination have also contributed to increased oxygen and mineral nutrient availability for the autochthonous microorganisms as a result of the increased microbial activity, increased growth of THDB and the increased TPH reduction that were observed. More also, both the plant and animal organic wastes microbial population supply was also relevant as it may provide additional hydrocarbon degrading microorganisms [67], which could contribute to metabolize hydrocarbon contaminant together with the soil autochthonous microorganisms.

### Conclusions

The present studies confirm that the use of crop residues and animal dung wastes (organic wastes) improved the rate of biodegradation in microcosms simulating soil or land environments contaminated with crude oil. The maximum total petroleum hydrocarbon (TPH) removal of 96.62 % and 94.86 % was obtained for the use of pig dung and cassava peels as biostimulant from the group of animal and crop residue organic wastes, respectively. Furthermore, the most efficient removal of TPH using the various organic wastes alone and/or in combinations occurred within the first 21 days. The biodegradation rate constant obtained from the application of first order kinetics described the rate of crude oil biodegradation with and without biostimulant. The rate constant ($k$) ranges between 0.0228 day$^{-1}$ and 0.0498 day$^{-1}$ for amended soil microcosm and 0.0144 day$^{-1}$ for unamended soil microcosm (natural attenuation). A half-life time ($t_{1/2}$) of 48.1 days was observed for biodegradation of crude oil in soil not amended with biostimulant. This was reduced to between 13.9 and 29.5 days with the usage of biostimulant in the form of crop residues and animal dung wastes (organic wastes). Statistical analysis using ANOVA and Tukey’s test to determine significance effect of the biostimulation agents on WBLCO biodegradation also showed that WBLCO biodegradation in soil was highly influenced by the different crop and animal-derived organic wastes and NPK fertilizer. There was a significant difference in the biostimulation efficiency of the different crop and animal-derived organic wastes (alone or combinations) as well as between the wastes and NPK fertilizer.

From the biostimulation efficiency (%B.E) and biodegradation rate constant ($k$) values, the performance of the animal dung and crop residue organic wastes used alone followed this decreasing order: PD>CP>CD>GS>MS>BS; while the performance of animal dung and crop residue organic wastes used at two level combinations followed this decreasing order:

$$PD+CP>GS+MS>CD+GS>BS+CD+MS+GS+CP>CD+CP>C$$

Thus, the bioremediation of WBLCO contaminated soil can be achieved by treating with crop and animal-derived wastes (PD, CD, BS, MS, CP, GS), and NPK fertilizer.
CP, GS, MS and BS) used singly or in combination. The bioremediation process used in this study was simple, inexpensive, efficient and environmentally compatible because by-product of animal and crops regarded as wastes and of no economic value was used to increase crude oil removal. The efficiency of the system depended on the organic nutrients as well as its source, PD, CD, BS, and CP being the most convenient and easily attainable. The bioremediation technique proposed here for soils contaminated with crude oil and other lighter oil distillates could be suitable in field, because of its low costs and its low environmental risk associated with volatile hydrocarbon losses.

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