Journal of Environment and Earth Science
ISSN 2224-3216 (Paper) ISSN 2225-0948 (Online)
Vol. 3, No.7, 2013

Application of Carbon-Nitrogen Supplementation from Plant and Animal Sources in *In-situ* Soil Bioremediation of Diesel Oil: Experimental Analysis and Kinetic Modelling

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The research is financed by the authors

Abstract
In this study, the potential effects of sawdust, yam peel and mixture of cow dung, goat dung and poultry dung used alone or in combination as amendment/nutrient supplements to biostimulate autochthonous microflora for hydrocarbon biodegradation were investigated in microcosms containing soil spiked with diesel oil (10 % w/w). The rates of biodegradation of the diesel oil were studied for 42 days remediation period under laboratory conditions. The results showed that there was a positive relationship between the microbial growth, biodegradation rate and presence of the sawdust, yam peel and the mixture of cow dung, goat dung and poultry dung (alone or in combination) in microcosms simulated diesel oil contaminated soil. The biodegradation data fitted well to first-order kinetic model. The model revealed that the combination of sawdust, yam peel, cow dung, goat dung and poultry dung elicited higher diesel oil biodegradation with biodegradation rate constant of 0.089 day^{-1} and half-life of 7.79 days. The system proposed here takes advantage of the organic wastes bulking properties as well as the autochthonous microorganism metabolic activity to efficiently degrade petroleum hydrocarbons. This system is inexpensive, efficient, and environmentally friendly and may thus offer a viable choice for petroleum hydrocarbons-contaminated soil remediation.
Keywords: Biodegradation; Biostimulation; Diesel oil; Organic wastes; First-order kinetics; Half-life.

1. Introduction

Petroleum-based products are the major source of energy for industry and daily life. Leakages and accidental spills occur regularly during the exploration, production, refining, transport, and storage of petroleum and petroleum products. The contamination of soil by these crude oil and petroleum products has become a serious problem that represents a global concern for the potential consequences on ecosystem and human health (Onwurah et al., 2007). Among petroleum products, diesel oil is a complex mixture of alkanes and aromatic compounds that are frequently reported as soil contaminants leaking from storage tanks and pipelines or released in accidental spills (Gallego et al., 2001). The scale of hazards imposed on the natural environment depends on the surface of the area contaminated by the petroleum products, their chemical composition, and the depth at which pollutants occur (Wolicka et al., 2009). The technology commonly used for soil remediation includes mechanical, burying, evaporation, dispersion, and washing. However, these technologies are expensive and can lead to incomplete decomposition of contaminants (Das and Chandra, 2011). For this reason an increasing attention has been directed toward the research of new strategies and environmental-friendly technologies to be applied for the remediation of soil contaminated by petroleum hydrocarbons. Among these, bioremediation technology which involves the use of microorganisms to detoxify or remove pollutants through the mechanisms of biodegradation has been found to be an environmentally-friendly, noninvasive and relatively cost-effective option (April et al., 2000). Diesel oil bioremediation in soil can be promoted by stimulation of the indigenous microbial population, by introducing nutrients and oxygen into the soil (biostimulation) (Seklemova et al., 2001) or through inoculation of an enriched microbial consortium into soil (bioaugmentation) (Richard and Vogel, 1999; Bento et al., 2005).

Following oil pollution, nutrients are rapidly assimilated by soil microorganisms thus depleting the nutrient reserves (Rahman et al., 2002). Therefore, apart from the environmental problem caused by oil pollution, the agronomic and economic aspects are significant (Jobson et al., 1974; Kuhn et al., 1998). The objective of using amendments is to augment the native fertility status of such soil and to enhance the rate of oil degradation, thus minimizing the contamination of scarce groundwater sources and to improve crop production (Amadi, 1990). The addition of inorganic or organic nitrogen-rich nutrients (biostimulation) is an effective approach to enhance the bioremediation process (Margesin et al., 2007; Abioye et al., 2009). Positive effects of nitrogen amendment using nitrogenous fertilizer on microbial activity and/or petroleum hydrocarbon degradation have been widely demonstrated (Akinde and Obire, 2008, Agarry et al., 2010a, 2010b).

In most soil bioremediation studies, inorganic chemical fertilizers have been widely used as biostimulating agent, however, it is relatively scarce and costly as well as not sufficient for agriculture due to high demand, let alone for cleaning oil spills (Agarry et al., 2010b; Danjuma et al., 2012). Therefore, the search for cheaper and environmentally friendly options of enhancing petroleum hydrocarbon degradation through biostimulation has been the focus of research in recent times (Agarry et al., 2010b; Danjuma et al., 2012; Nyankanga et al., 2012). One of such option is the use of organic wastes derived from plant and animals. Few workers have investigated the potential use of plant organic wastes such as rice husk and coconut shell (Nyankanga et al., 2012), plantain peels and cocoa pod husk (Agbor et al., 2012), molinga oleifera and soya beans (Danjuma et al., 2012) and animal organic wastes like cow dung, pig dung, poultry manure and goat dung (Yakubu, 2007; Adesodun and Mbagwu, 2008; Agarry et al., 2010a; Agarry and Ogunleye, 2012) as biostimulating agents in the cleanup of soil contaminated with petroleum hydrocarbons and were found to show positive influence on petroleum hydrocarbon biodegradation in a polluted environment. Nevertheless, the search for cost effective and environmentally friendly methods of enhancing petroleum hydrocarbon biodegradation in soil still needs to be further investigated. To the best of our knowledge, information on the use of cellulose or starch waste from plant source such as sawdust and yam peel as well as the combined mixture of cow dung, poultry dung and goat dung as amendment agents for the stimulation of autochthonous microflora of petroleum hydrocarbons-contaminated soils is relatively limited.

The integration of mathematical modeling and experimental testing is a key issue for a better comprehension and prediction of bio-treatments efficiency devoted to hydrocarbon removal in contaminated soil. Kinetic models can be a useful tool for the prediction of residual contaminant concentrations during bioremediation (Venosa and Holder, 2007; Bayen et al., 2009). Kinetic models of growth of natural bacterial assemblages during bio-treatments of contaminated soil received little attention, but these can be a relevant support for a better understanding of biodegradation rates of hydrocarbons and prediction of residual contaminant concentrations.

In this study we carried out bioremediation experiments on soil contaminated by diesel oil, investigating the effects of sawdust and yam peel (plant source waste) and the mixture of cow dung, pig dung and goat dung (animal source waste) as biostimulating/amendment agents on the kinetics of microbial hydrocarbon degradation. Experimental results were then used to assess the suitability of a rather simple first-order empirical model to predict changes in residual hydrocarbon concentrations during bio-treatments in order to provide a support tool when designing bioremediation strategies on site.

2. Materials and Methods

2.1 Collection of Samples

The soil sample used for the study was collected from the top surface soil (0 – 15cm) 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 diesel oil was obtained from petroleum products station, Ogbomoso, Nigeria. The cow dung (CD) and goat dung (GD) was collected from a cow market and goat market respectively, in Ogbomoso, Nigeria. The pig dung (PD) was obtained from the piggery farm of LAUTECH, Ogbomoso, Nigeria. The yam peel (YP) was obtained from a local restaurant in Tarki Area of Ogbomoso, Nigeria. The sawdust (SD) was obtained from a furniture company in Ogbomoso, Nigeria. All the different amendment agents were each sun dried for two weeks, grinded and sieved to obtain uniform size particles. Each amendment agent was stored in a polyethylene bag and kept prior to use.

2.2. 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 (1982); total organic carbon was determined by the modified wet combustion method (Nelson and Sommers, 1982) and total nitrogen was determined by the semi-micro-Kjeldhal method (Bremner and Mulvaney, 1982). Available phosphorus was determined by Brays No.1 method (Olsen and Sommers, 1982) and moisture content was determined by the dry weight method. The total hydrocarbon degrading bacteria (THDB) populations was determined by the vapor phase transfer method (Amanchukwu et al., 1989). The physicochemical characterized parameters are presented in Table 1.

Table 1. Soil sample and organic wastes physicochemical and microbiological analysis

Parameter	**Soil**	**SD**	**YP**	**PD**	**GD**	**CD**
Organic carbon (%)	1.05±0.01	49.7±0.3	37.6	24.3 ± 0.02	22.2 ± 0.01	26.3 ± 0.01
Total nitrogen (%)	0.77±0.03	2.5	1.28 ± 0.02	2.70 ± 0.02	2.10 ± 0.03	2.40 ± 0.01
Carbon: Nitrogen	1.4:1	20:1	29:1	9:1	11:1	11:1
Phosphorus (%)	0.06 ± 0.02	0.035	0.044 ± 0.03	0.39 ± 0.01	0.23 ± 0.02	0.25 ± 0.03
pH	7 ± 0.1	5.2 ± 0.3	6.5 ± 0.2	7.2 ± 0.2	6.9 ± 0.1	8.4 ± 0.2
Moisture (%)	11.4 ± 0.02	9.4 ± 0.1	7.2 ± 0.2	5.0 ± 0.3	6.3 ± 0.2	8.4 ± 0.2
THDB (CFU/g)	$0.1±0.8×10^4$	$0.1±0.1×10^3$	$0.2±0.2×10^3$	$0.3±2.3×10^5$	$0.1±0.7×10^5$	$0.3±1.7×10^5$

Note. Each value is a mean of three replicates and ± indicates standard deviation among them.
SD = Sawdust; YP =Yam peel; PD = Poultry dung; GD = Goat dung; CD = Cow dung

2.3. Solid-Phase Experimental Design and Soil Treatment

Soil samples (1 kg) was put into 8 different plastic bins (microcosm) with a volume of about 3 L and labeled A to H, respectively. The soil in each plastic bins was spiked with 10% (w/w) diesel oil 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 (Osuji et al., 2005). Two weeks after contamination, the different remediation treatments were applied. The soil C:N ratio in each microcosm was adjusted by the addition of sawdust (SD), yam peel (YP), mixture or combination of cow dung (CD), goat dung (GD) and poultry dung) respectively, as carbon co-substrate and nitrogen source and was thoroughly mixed (Table 2). It was assumed that the aforementioned quantities of the plant-residue organic wastes and animal-derived organic wastes applied to the relevant treatment microcosm 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 tilled twice a week for aeration, and the moisture content was maintained at 50% water holding capacity. The soil in Plastic bin A was autoclaved three times at 121°C for 30 min before contamination with diesel oil. The contaminated autoclaved soil in plastic bin A was without amendment agents and thus served as control. The experiment was set up in triplicate. In total, 24 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) and hydrocarbon degrading bacteria, respectively.

Table 2. Experimental design and types of organic wastes combination in different soil microcosm

Microcosm Code	**Remediation Treatment and Description**	**C:N**
A	1 kg Autoclaved Soil + 100 g Diesel oil	-
B	1 kg Soil + 100 g Diesel (Natural Attenuation)	-
C	1 kg Soil + 100 g Diesel + 75 g SD	46:1
D	1 kg Soil + 100 g Diesel + 75 g YP	89:1
E	1 kg Soil + 100 g Diesel + 25 g CD + 25g GD + 25 g PD	16:1
F	1 kg Soil + 100 g Diesel + 37.5 g SD + 37.5g YP	10:1
G	1 kg Soil + 100 g Diesel + 15 g SD + 15 g YP + 15 g CD + 15 g GD + 15 g PD	30:1
H	1 kg Soil + 100 g Diesel oil + 20 g of NPK fertilizer(20:10:10)	20:1

2.4 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 (2008). Soil samples (approximately 10 g) 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 minutes 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 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 in soil 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:

$$D = \frac{TPH_i - TPH_r}{TPH_i} \times 100 \qquad (1)$$

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

2.5 Data Analysis

The data were analyzed for significant differences at the level of $p < 0.05$ between treatments using one- way analysis of variance (ANOVA) tests which were performed using statistical package for social sciences, version 16.0 (SPSS Inc., Chicago, IL, USA).

2.6 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 (Pala et al., 2006; Agarry et al., 2010b; Zahed et al., 2011) and this is given as in Eq. (2):

$$C_t = C_o e^{-kt} \qquad (2)$$

Where C_o 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.

2.7 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 (Aroson et al., 2006), environmental fate modeling (Sinkkonen and Paasivirta, 2000) and describing the transformation of pollutants (Dimitrov et al., 2007; Matthies and Klasmeier, 2008). Biodegradation half life times ($t_{1/2}$) are calculated by Eq. (3) (Yeung et al., 1997; Zahed et al., 2011; Agarry et al., 2013):

$$t_{1/2} = \frac{\ln 2}{k} \qquad (3)$$

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. Results and Discussion

3.1 Natural Attenuation and Biostimulation

The level of diesel oil biodegradation in soil amended with plant residues (sawdust and yam peel) and animal dung wastes (mixture of cow dung, goat dung and poultry dung)) either alone or in combination is shown in Figure 1.

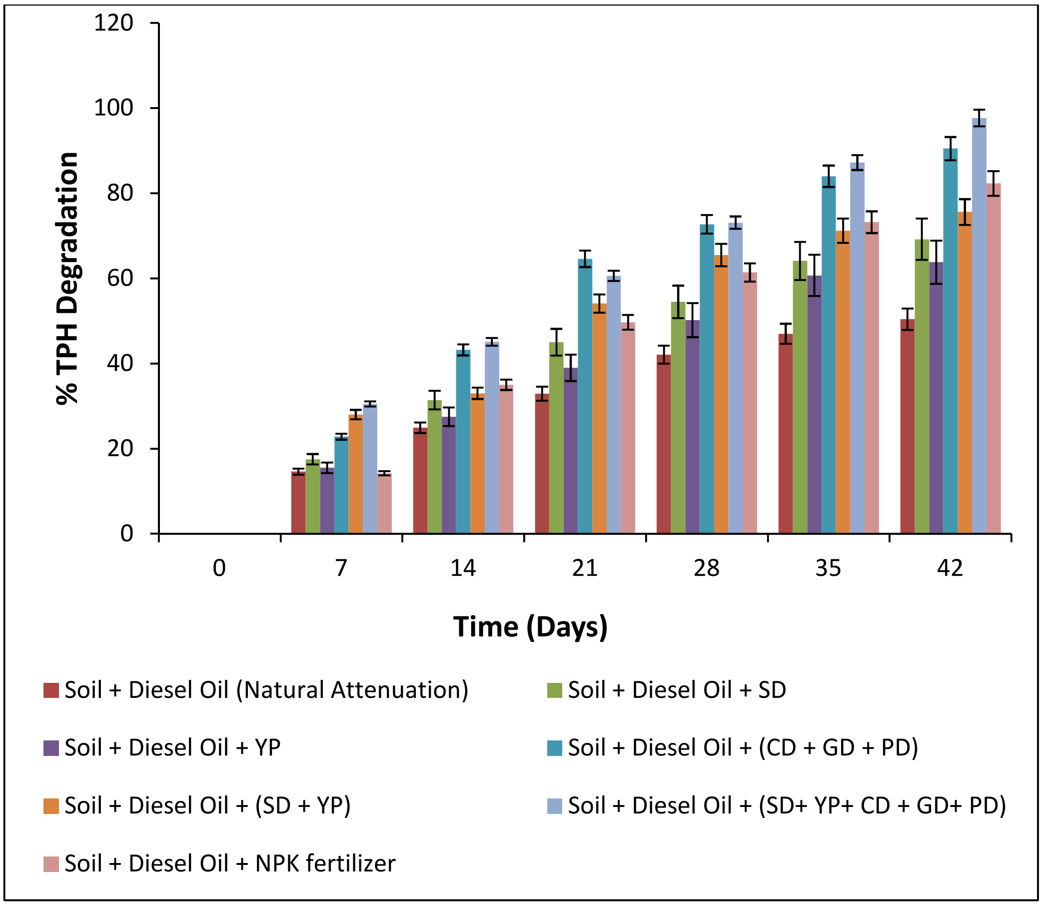

Figure 1. Time course for the biodegradation of diesel oil in soil microcosms amended with SD, YP, CD + GD + PD, SD + YP, SD + YP + CD + GD + PD, NPK, and in unamended soil microcosm (natural attenuation). Bars indicate the average of triplicate samples while the error bars show the standard deviation.

It is observed that the percentage reduction in TPH was rapid within the first 28 days of the study in all the soil amended with plant residues, animal dung wastes and NPK fertilizer when compared to that of the unamended soil microcosm. At the end of day- 28, there was 54.5%, 50.2%, 72.7%, 65.5%, 73.1% and 61.4% TPH reduction in soil microcosms C, D, E, F, G and H amended with SD, YP, CD + GD + PD, SD + YP, SD + YP + CD + GD + PD and inorganic NPK fertilizer, respectively; while 42.1% TPH reduction occurred in the un-amended soil microcosm B (natural bioattenuation). At the end of remediation period (day-42), diesel oil-contaminated soil amended with the combination of SD + YP + CD + GD + PD showed the highest reduction in TPH concentration (97.7%), relatively followed by soil microcosms amended with CD + GD + PD (90.5%), NPK fertilizer (82.3%), SD + YP (75.6%), SD (69.2%), and YP (63.8%) while the un-amended soil (natural bioattenuation) showed 50.4% reduction at the end of day-42.

These observations indicate that the sawdust and yam peel (plant source waste) and the mixture of cow dung, goat dung and poultry dung (animal source waste) used alone and/or in combination enhanced diesel biodegradation in soil. Similar observations have been reported for the use of plant and animal-derived organic waste (Liu et al., 2010; Akpoveta et al., 2011) in the bioremediation of soil contaminated with petroleum hydrocarbons. Liu et al. (2010) used organic manure made up of rice straw and pig dung to biostimulate the degradation of an oily sludge and obtained a TPH reduction of 58.2% in a remediation period of 360 days, while Akpoveta et al. (2011) made use of the mixture of cow dung, pig dung and poultry dung to biostimulate crude oil biodegradation in soil and obtained 81.7% TPH reduction in a remediation period of six weeks. Addition of nutrients including nitrogen (N) and phosphorus (P) is standard practice for increasing hydrocarbon degradation (Atlas and Bartha, 1998). 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. Mills and Frankenberger (1994) evaluated the effect of phosphorus sources and concentration (100 – 1 000 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. Knowledge of bioavailability of nutrients is necessary in the planning of an efficient bioremediation strategy.

Figure 2 shows the growth profiles of the total hydrocarbon degrading bacteria (THDB) in microcosms due to natural attenuation and biostimulation treatment methods. Generally, it is seen that the microbial (THDB) counts increased from day 0 to day 42 in each of the treatment microcosms. For microcosm T3 (natural attenuation), the THDB count increased from 0.02±2.2 to 0.04±6.7 × 10^6cfu-g^{-1}, while it increased from 0.03±2.10 to 0.02±10.0 × 10^6cfu-g^{-1}, 0.03±2.20 to 0.02±9.20 × 10^6cfu-g^{-1} , i0.03±2.40 to 0.02±14.60 × 10^6cfu-g^{-1} , 0.03±2.10 to 0.02±10.80 × 10^6cfu-g^{-1} , 0.03±2.50 to 0.02±16.0 × 10^6cfu-g^{-1} , and 0.03±2.3 to 0.02±12.6 × 10^6cfu-g^{-1} for soil microcosm C, D, E, F, G and H amended with SD, YP, CD + GD + PD, SD + YP, SD + YP + CD + GD + PD and NPK fertilizer, respectively.

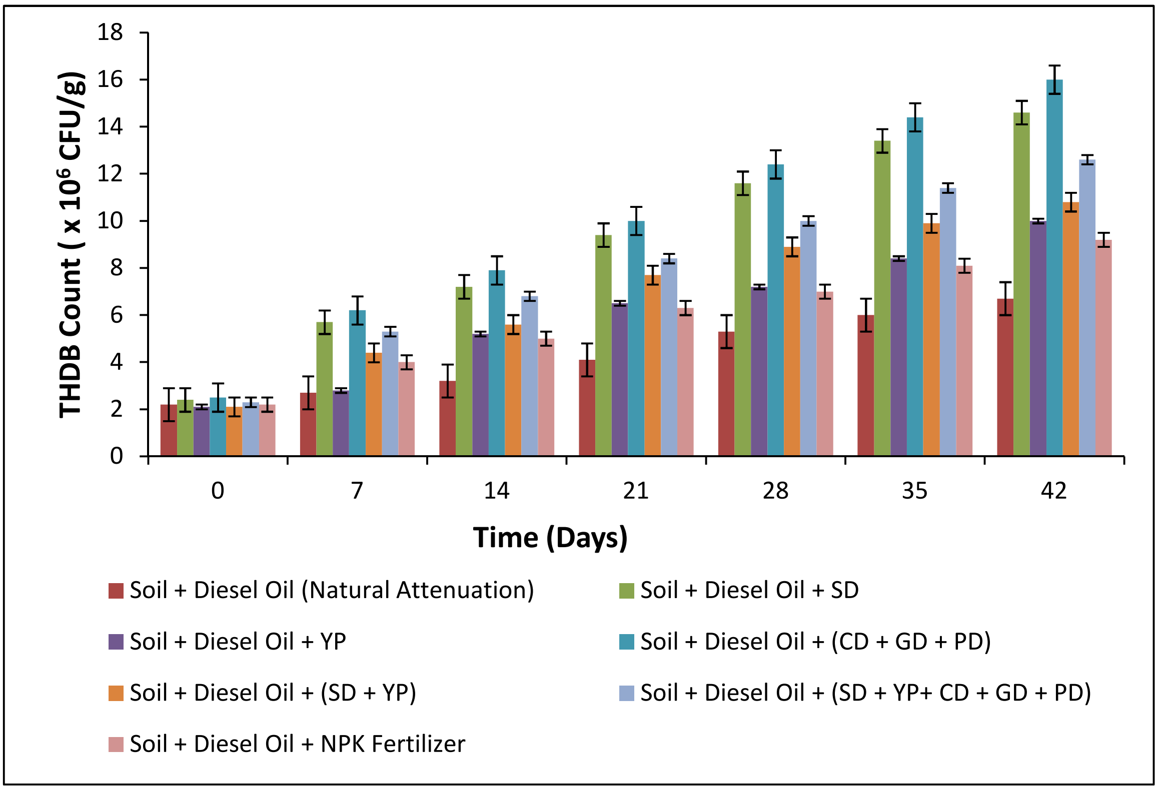

Figure 2. Time course for the growth of THDB on diesel oil in soil microcosms amended with SD, YP, CD + GD + PD, SD + YP, SD + YP + CD + GD + PD, NPK, and in unamended soil microcosm (natural attenuation). Bars indicate the average of triplicate samples while the error bars show the standard deviation.

This corresponded to a growth increase of 79%, 76%, 83.6%, 80.6%, 84.4% and 81.7% for soil microcosms C, D, E, F, G and H, respectively. The percentage THDB growth in the unamended soil (natural attenuation) is 67.2%. This showed that the soil microcosms amended with the biostimulation agents: SD, YP, mixture of CD, GD and PD, and inorganic NPK fertilizer enhanced the microbial growth rate which accounted for the higher microbial counts observed in all the amended soil microcosms than the unamended soil microcosm (natural attenuation). Similar observations have been reported for the use of starch, glucose, crop and animal organic waste in the biodegradation of petroleum hydrocarbons in soil (Teng et al., 2010; Ibiene et al., 2011; Agbor et al., 2012; Nduka et al., 2012). The higher microbial count in amended soil microcosms (biostimulation) may be due to high nutrient level which stimulated increase in microbial population and activities thus leading to high energy (carbon) demand by the oil-degrading microbes. This has resulted in the increased reduction of total petroleum hydrocarbon (TPH) in the amended soil microcosms.

3.2 Evaluation of Biodegradation Kinetics and Half-Life

First-order kinetics model equation (Eq. 3) fitted to the biodegradation data was used to determine the rate of biodegradation of diesel oil in the various remediation treatments which is illustrated in Figure 3.

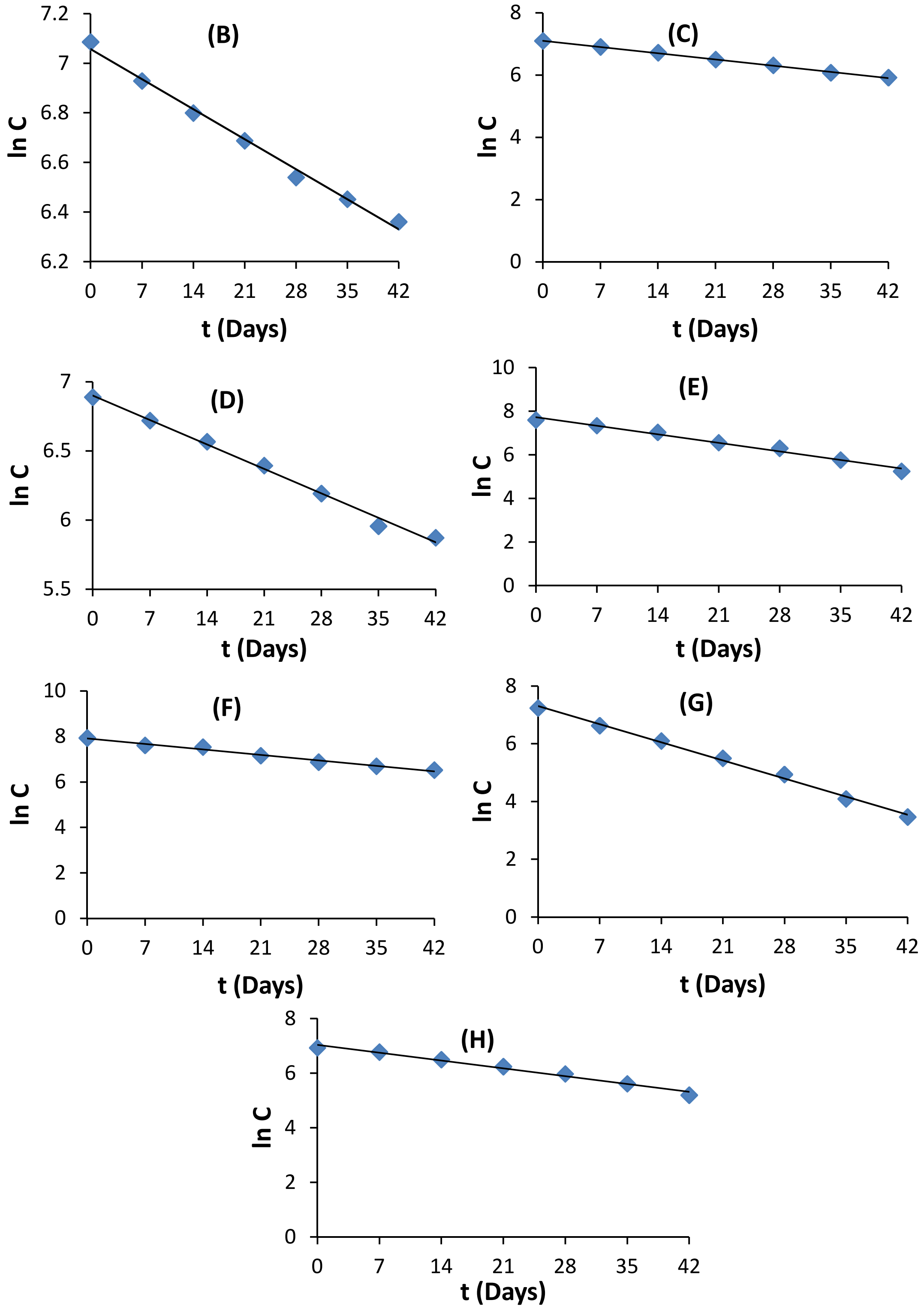

Figure 3. First-order kinetic model fitted to biodegradation data of diesel oil in unamended soil microcosm (B) natural attenuation, and soil microcosm amended with SD (C), YP (D), CD + GD + PD (E), SD + YP (F), SD + YP + CD + GD + PD (G), NPK fertilizer (H).

The biodegradation data fitted well to the first-order kinetic model with high correlation coefficient that lies between 0.98 and 0.99. The half-life times of diesel 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 3.

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

Microcosm code	Biostimulation treatment	k (day^{-1})	R^2	$t_{1/2}$ (days)
B	Unamended soil	0.017	0.992	40.8
C	Sawdust	0.028	0.998	24.8
D	Yam peel	0.025	0.993	27.7
E	Cow dung, goat dung, poultry dung	0.055	0.985	12.6
F	Sawdust, yam peel	0.034	0.984	20.3
G	Sawdust, yam peel, Cow dung, goat dung, poultry dung	0.089	0.995	7.79
H	NPK fertilizer	0.041	0.983	16.9

Same small letters indicate that treatments are statistically ($p < 0.05$) not different.

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 soil microcosms amended with SD, YP, mixture of CD, GD and PD, and inorganic NPK fertilizer, the soil microcosm amended with the combination of plant and animal-source organic waste, SD + YP + CD + GD + PD had a higher biodegradation rate constant k (0.089 day^{-1}) and lower half-life time ($t_{1/2}$ =7.79 days) than others. However, this was relatively followed by soil microcosms amended with the mixed animal source waste, CD + GD + PD (k = 0.055 day^{-1} and $t_{1/2}$ = 12.6 days), NPK fertilizer (k = 0.041 day^{-1} and $t_{1/2}$ = 16.9 days), SD + YP (k = 0.034 day^{-1} and $t_{1/2}$ = 20.3 days), SD (k = 0.028 day^{-1} and $t_{1/2}$ = 24.8 days), and YP (k = 0.025 day^{-1} and $t_{1/2}$ = 27.7 days), respectively. The biodegradation rate constant (k) and half-life time ($t_{1/2}$) for the un-amended soil microcosm (natural attenuation) was obtained to be 0.017 day^{-1} and 40.8 days, respectively.

Thus, the biodegradation rate constants obtained for the different diesel soil contaminated microcosms amended with the plant and animal-source organic wastes either alone or in combinations were higher with lower half-life times than that of the unamended soil microcosm (natural attenuation). Moreover, the biodegradation rate constants obtained for the soil microcosms amended with sawdust and yam peel (SD and YP) alone or in combination were relatively lower than that obtained for soil microcosm amended with the inorganic NPK fertilizer. Nevertheless, these observations indicate that the addition of sawdust and yam peel (plant source waste) and the mixture of cow dung, goat dung and poultry dung (animal source waste) alone or in combinations enhanced TPH reduction. However, the variations in the rate constants and half-life times observed in the different treatments may be due to the different carbon: nitrogen ratio (C:N) provided by the animal and plant organic wastes into the soil (Table 1). Teng et al. (2010) have reported that soil amendment with the addition of starch, glucose and sodium succinate enhanced the biodegradation of phenanthrene and benzo(pyrene) and that the soil amendment with C/N ratio of 10:1 significantly elicited higher polycyclic aromatic hydrocarbon biodegradation than those with C/N ratio of 25:1 and 40:1, respectively.

3.3 Effectiveness of Biostimulation Supplements

A one-way ANOVA analysis was conducted to compare the biodegradation efficiency of the biostimulation or amendment agents and the result is presented in Table 4.

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	945016.5	6	157502.7	4524	0.0000
Error	487.33	14	34.809		
Total	945503.83	20			

The result suggests that the biostimulation or amendment agents had a statistically significant effect on the biodegradation of diesel oil 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 day-42 remediation period using Eq. (5) (Zahed et al., 2011):

$$\% \text{B.E} = \frac{\%TPH_{(S)} - \%TPH_{(U)}}{\%TPH_{(S)}} \times 100 \quad (5)$$

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 21 and 48.4%. The results in Table 5 generally showed that there are relative variations in the biostimulation efficiency of the plant and animal-derived organic wastes.

Table 5. Percent degradation of diesel oil and biostimulants efficiency at the end of six weeks

Microcosm code	Biostimulation treatment	% degradation	BE (%)
B	Unamended soil	50.4 ± 0.05	-
C	Sawdust	69.2 ± 0.03	27.2
D	Yam peel	63.8 ± 0.01	21.0
E	Cow dung, goat dung, poultry dung	90.5 ± 0.02	44.3
F	Sawdust, yam peel	75.6 ± 0.03	33.3
G	Sawdust, yam peel, Cow dung, goat dung, poultry dung	97.7 ± 0.02	48.4
H	NPK fertilizer	82.3 ± 0.01	38.8

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.

Table 6. Grouping of TPH mean for the different treatments computed by Tukey's method

Treatments	TPH Mean (mg/kg)	Standard error
Natural attenuation	579^{B}	5.19
Soil + Diesel + SD	372^{C}	3.46
Soil + Diesel + YP	355^{D}	3.18
Soil + Diesel + CD + GD + PD	190^{E}	4.33
Soil + Diesel + SD + YP	681^{A}	2.33
Soil + Diesel + SD + YP + CD + GD + PD	35.3^{G}	2.40
Soil + Diesel + NPK fertilizer	181.3^{F}	1.45

Means that do not share the same letter are significantly different

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 NPK fertilizer, the sawdust and yam peel (plant source waste), and mixture of cow dung, goat dung and poultry dung (animal source waste) alone or in combination. It further showed that there are significant differences in the biostimulation efficiency of sawdust, yam peel and the mixture of cow dung, goat dung and poultry dung.(alone or in combination).

Therefore, between the different plant and animal-source organic waste (SD, YP, mixture of CD, GD and PD) used alone or in combination, the combination of sawdust, yam peel, cow dung, goat dung and poultry dung (i.e. SD + YP + CD + GD + PD) suggests to be relatively more effective with higher B.E (48.4%) than others. This is relatively followed by the mixture of cow dung, goat dung and poultry dung (i.e. CD + GD + PD) (44.3%), SD + YP (33.3%), SD (27.2%) and YP (21%), respectively. Mean while, the B.E for inorganic NPK fertilizer is 38.8%. The results in this study suggests that combination of several animal dung wastes and as well as combination of animal dung wastes and plant/crop residue organic wastes has a 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. (2010b). 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 sawdust, yam peel, mixture of cow dung, goat dung and poultry dung used alone and/or in combination as biostimulant in the enhancement of diesel 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 plant residues as well as in the animal dung wastes may be important factors which regulate microorganism growth and activity (Cookson et al., 1998). Furthermore, Molina-Barahona et al. (2004) 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 has been reported for the use of sawdust in the removal of oil and grease in a contaminated soil (Elektorowicz, 1994).

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 (Elektorowicz, 1994; Piehler et al., 1999; Jørgensen et al., 2000; Molina-Barahona et al., 2004). Thus, in our system, the results suggested that both plant organic wastes (sawdust and yam peel) and animal dung wastes (mixture of cow dung, goat dung and poultry dung) used 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 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 (Jørgensen et al., 2000), which could contribute to metabolize hydrocarbon contaminant together with the soil autochthonous microorganisms.

Conclusions

The present studies confirm that the use of plant residues such as sawdust and yam peel, and animal dung wastes (mixture of cow dung, goat dung and poultry dung) used alone or in combination improved the rate of petroleum hydrocarbon biodegradation in contaminated soil microcosms. The biodegradation rate constant obtained from the application of first order kinetics described the rate of diesel oil biodegradation with and without biostimulant. The rate constant (k) ranges between 0.025 day^{-1} and 0.089 day^{-1} for amended soil microcosm and 0.017 day^{-1} for unamended soil microcosm (natural attenuation). A half-life time ($t_{1/2}$) of 40.8 days was observed for biodegradation of diesel oil in soil not amended with biostimulant. This was reduced to between 7.79 and 12.6 days with the use of biostimulant in the form of plant residues (sawdust and yam peel) and animal dung wastes (mixture of cow dung, goat dung and poultry dung). 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 and in combination as well as the NPK fertilizer follows this decreasing order: SD + YP + CD + GD + PD > CD + GD + PD > NPK fertilizer > SD + YP > SD > YP. The bioremediation technique proposed here for soils contaminated with diesel oil and other petroleum hydrocarbons could be suitable in field, because of its low costs and its low environmental risk associated with volatile hydrocarbon losses. However, the success and efficiency of these biostimulation techniques may vary considerably from one site to another; hence, there is no universal soil treatment regimen for the bioremediation of all petroleum hydrocarbon-contaminated soils. The effectiveness of any soil treatment applied for such purpose has to be evaluated on a case-specific basis. Nevertheless, two important issues need to be addressed before taking such technology to the field (Agarry and Ogunleye, 2012): (1) evaluation of the intrinsic microbial population and its metabolic potential and (2) evaluation of the environment where such bioremediation process is needed for lack of essential nutrients and the residual oil concentration.

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