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Optimization of Diesel and Crude Oil Degradation in a Ghanaian Soil Using Organic Wastes as Amendment

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Abstract

Soil contamination by hydrocarbons poses numerous environmental, health and agricultural problems. The degradation of these pollutants can occur naturally but very slowly. It is therefore generally necessary to stimulate this degradation by different means. Thus, this study aimed to improve the biodegradation of diesel and crude oil in a Ghanaian soil by biostimulation. For this, the sampled soil was characterized by standard methods and contaminated with diesel and crude oil at a proportion of 1% (w/w). Then, contaminated soil samples were supplemented with biochar-compost, poultry manure or cow dung at the proportion of 10% (w/w). Periodically, fractions of these samples were taken to evaluate the density of hydrocarbon utilizing bacteria (HUB) and the residual quantities of diesel or crude oil. The characteristics of the soil used show the need for supplementation for better degradation of hydrocarbons. The results of the study show that supplementing the soil with organic substrates increases HUB loads in soils contaminated by diesel and crude oil. They also show that the residual quantities of diesel and crude oil are generally significantly lower in supplemented soils ($p = 0.048$ and $p < 0.0001$ respectively). In addition, the study shows that degradation was generally greater in soils contaminated by diesel compared to those contaminated by crude oil, especially at the end of the study.

Keywords

Biodegradation, Biostimulation, Soil, Diesel, Crude Oil, Organic Amendment, Ghana

1. Introduction

Crude oils and diesel are pollutants that can lead to environmental, health and agricultural problems [1] [2]. Once hydrocarbons contaminants enter the soil, they pose severe threats to humans, animals, and plants [3]. In addition, the presence of petroleum hydrocarbons affects the physical, chemical and ecological properties of soil [4] [5]. Hydrocarbons are present in soil all over the world and because of their low volatility and hydrophobic nature, they persist in polluted environments [6]. Many solutions are developed around the world to degrade these pollutants. These solutions may be physical [7], chemical [8] or biological [9] and are generally complementary. According to Qinglong *et al.* [10] and Bianco *et al.* [11], physical and chemical methods of degrading petroleum products are costly and time consuming. The use of these methods also leads to waste-producing [12]. Thus, bioremediation involving the microorganisms in the environment remains the most effective alternative for degradation of hydrocarbons [13] [14]. However, several factors influence the biodegradation of hydrocarbons in natural environments and may be in some cases, a limiting factor. So, lack of essential nutrients such as nitrogen and phosphorus is an important factor affecting microbial biodegradation of hydrocarbons in polluted environments [15] [16]. Several studies have shown that intake of organic wastes containing these two nutrients can stimulate the biodegradation of hydrocarbons [17] [18]. Organic amendments not only supply a source of carbon and nutrients but also add exogenous beneficial microorganisms to enhance hydrocarbons degradation rate, thereby improving the soil health [19]. However, the good effect of biostimulation on soil hydrocarbons biodegradation is not always obvious for a number of reasons. So, according to Hoang *et al.* [19], net immobilization and mobilization of soil hydrocarbons have both been observed following the application of organic amendments to contaminated soils. These observations show that the effects of organic wastes on biodegradation of hydrocarbons in soils might be dissimilar [20]. This means that it is necessary to continue to test, for each type of soil, the possible effect of an organic amendment on the biodegradation of hydrocarbons. So, the present study aims to optimize the biodegradation of diesel and crude oil in soil of Ghana by biostimulation with organic amendment.

2. Materials and Methods

2.1. Samples Collection

The soil sample used in this study was collected in the garden of University of Ghana (5.65978N, 0.193269E). This soil had no known history of diesel or crude oil contamination. The plough layer (0 - 15 cm) of the soils was taken in different areas and mixed, to obtain a single soil sample. The soil sample was air dried during a week and sieved (mesh size of 2 mm).

Cow dung and poultry manure were collected from different areas of Ghana while biochar-compost was obtained from University of Ghana. All the organic amendments were air dried and sieved as for soil sample.

Diesel used in this study was purchased from a local oil filling station and stored in dark at ambient temperature throughout the study.

The crude oil used in this study was obtained from Jubilee Oil Field at Cape Three Point, Western Region of Ghana through the National Petroleum Authority.

2.2. Soil Sample Characterization

The pH and electrical conductivity were determined with pH meter (HANNA HI 8424) on 1:2.5 (w/v) soil/distilled water after 30 minutes equilibration. Triplicate determination was been made.

Total organic carbon was analyzed by potassium dichromate oxidation, as described by Schnitzer [21].

Soil available phosphorus was assessed according to the following method. 2 g of dry soil was extracted with 40 mL 0.5 M $NaCO_3$ at pH 8.5 after shaking for 30 min at 60 rpm. After centrifugation, the extract was analyzed and quantified using the molybdenum blue method for orthophosphate on an autoanalyzer.

Soil nitrogen was measured using 1:8 (w/v) 0.5 M K_2SO_4/distilled water as extractant. Then, the extracts were centrifuged at 60 rpm for 45 min and filtered. The extracts were subjected to total N analysis via a TOC-V CSN autoanalyzer (Shimadzu).

2.3. Optimization of Diesel and Crude Oil Degradation

To test the effect of organic wastes on diesel and crude oil biodegradation in soil, 200 g of each polluted soil (uncontaminated soil supplemented with 1% (w/w) of diesel or crude oil) were supplemented with 10% (w/w) of each organic amendment (poultry manure, cow dung, or biochar-compost) and thoroughly mixed in a plastic vessel. The contaminated soils were maintained under same moisture content (10% w/w basis) and incubated in dark at room temperature. The content of each vessel was tilled once a week for aeration and the moisture maintained at 10% w/w by the addition of distilled water. Treatment with only soil and diesel or crude oil served as control. All the experiments were done in triplicate. Regularly, Samples were taken at an interval of 10 days to assess the load of hydrocarbon utilizing bacteria and residual amount of diesel or crude oil.

2.4. Assessment of Load of Hydrocarbon Utilizing Bacteria in Soil

HUB load was determined at each 10 days for all the previous experiments. Thus, each time, 10 g of each culture were taken and introduced into a bottle containing 90 mL of sterile physiological water. The solution obtained was diluted in cascade. For each experiment, 1 mL of appropriate culture dilution was then taken and poured in agar plate containing Bushnell-Hass media supplemented with 20% agar-agar. The surface of agar plate was poured with 300 µL of diesel or crude oil after setting and allowed the diesel or crude oil to diffuse into the agar medium for about 2 hours before incubated at 37˚C for 5 to 7 days. HUB load was then determined and expressed as colony forming unit per milliliter (CFU mL^{-1}).

2.5. Residual Diesel and Crude Oil Extraction and Quantification

At each 10 days, composite samples were obtained by mixing 5 g of soil collected from different areas of plastic vessels of each culture and use. Residual diesel or crude oil in the contaminated soils was extracted using a modified method of Abu and Ogiji [22]. So, 5 g of soil were placed into plastic container and 10 mL chloroform were added. Residual oil was extracted by gently shaking the flask for 5 min at 150 rpm using an orbital shaker. Each extract was filtered through cotton wool in a funnel and collected in a clean glass container, closed immediately and analyzed for diesel or crude oil content. Quantitative determination of diesel or crude oil extracts was employed as described by Udeme and Antai [23]. Two standard curves of absorbance (520 nm) against varying concentrations of diesel or crude oil in chloroform were drawn after taking readings from a spectrophotometer (Pharo 300). Diesel or crude oil concentrations were calculated from the standard curves.

2.6. Statistical Analyzes

The data collected from biodegradation experiments were subjected to analysis of variance (ANOVA) with regards to petroleum product used, organic amendment and incubation time using XLSTAT-Pro 7.5 software. Mean variables were compared using the Newman Keuls test at probability level $p = 0.05$.

3. Results and Discussion

3.1. Soil Characteristics

The characteristics of the soil used for biodegradation are presented in **Table 1**. The pH and electrical conductivity of the soil amended with the organic wastes used in this study are presented in **Table 2**. The results presented in the **Table 1** show that the soil has a relatively acidic pH (4.6). The pH of the soil studied is relatively low compared to the pH 7.88 reported by Wu *et al.* [16] and those reported by Douglas *et al.* [24] which was 5.90. The electrical conductivity was similar to those reported by Curiel-Alegre *et al.* [25] (0.839 dS/m). The available phosphorus content of the soil studied was similar to that of Zhang *et al.* [26]

Table 1. Soil characteristics.

Main characteristic	Value
pH	4.6
EC	87.58 mS/m
Organic carbon	11.20 g/kg
Total Nitrogen	1.12 g/kg
Available Phosphorus	6.7 mg/kg

Table 2. pH and electrical conductivity of amended soil.

Type of soil	pH	EC (mS/m)
Soil + biochar-compost	7.90	4.996
Soil + cow dung	8.02	2.095
Soil + poultry	7.61	8.971

(12.1 mg/kg) and lower than that of Hussain *et al.* [27] (14.7 mg/kg). The nitrogen content measured on the soil studied was around 1.12 g/kg. This content is lower than that reported by Zhang *et al.* [26] which was 47 g/kg. The organic carbon content of the soil used in this study is similar to that of the soil used in a biodegradation experiment by Zhang *et al.* [26] of the order of 9.85 g/kg.

The C/N/P ration of the studied soil was of the order of 100/10/0.06, which differs from the optimal ratio of 100/10/1 recommended for better biodegradation of hydrocarbons in soil [28]. Thus, the amendment of this soil can be useful in a perspective of improving this ratio and then hydrocarbon degradation. In addition, the soil studied has an acidic pH which is not favorable for microbial growth and therefore biodegradation. So, an amendment of this soil could lead to a pH which would be close to the limit indicated for better degradation (6 to 7) [24].

3.2. Evolution of Hydrocarbon Utilizing Bacteria Density in Soil

In the case of diesel, the results obtained during this study show a fluctuating HUB density over time. The maximum HUB density is observed on the soil amended with biochar-compost. Throughout the experiment, the HUB loading of the amended soil is always higher than that of the unamended soil as shown in **Figure 1**. For crude oil, HUB loads are maximum on the 10th day and tend to decrease slightly thereafter. It is also observed that the microbial load is always higher in amended soils (**Figure 2**).

Thus, in general, for both types of substrates, the HUB loads are always greater in amended soils. This observation has already been revealed by other authors. Thus, according to Williams *et al.* [29], the number of HUB increases regularly during the biodegradation of diesel from contaminated soil in Nigeria. In addition, the number of HUB in the soil amended with cow dung and goat manure was always higher than the number of HUB in the unamended soil according to the same authors. Similarly, Douglas *et al.* [24], observed an evolution in the number of HUB similar to that of this study with an ever-increasing number of HUB in unamended soil during PAH biodegradation in Nigeria. According to Curiel-Alegre *et al.* [25], samples with vermicompost displayed the highest values of microbial biomass till the end of the incubation during hydrocarbons biodegradation in soil.

The greater presence of HUB in the amended soils for both cases (Diesel and crude oil) could be explained by a possible stimulation of HUB growth due to an improvement in soil properties linked to the presence of organic substrates. Indeed,

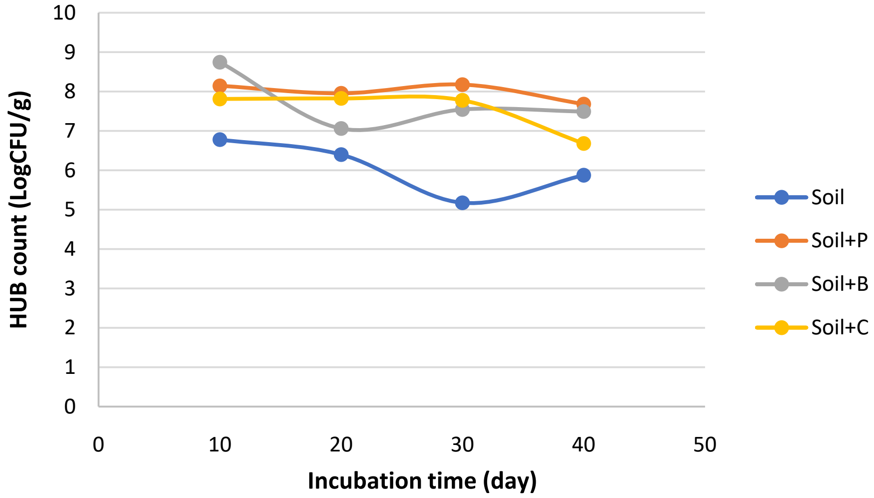

Figure 1. HUB loads in soils amended or not and supplemented with diesel. Legend: P: Poultry manure, B: biochar-compost, C: cow dung.

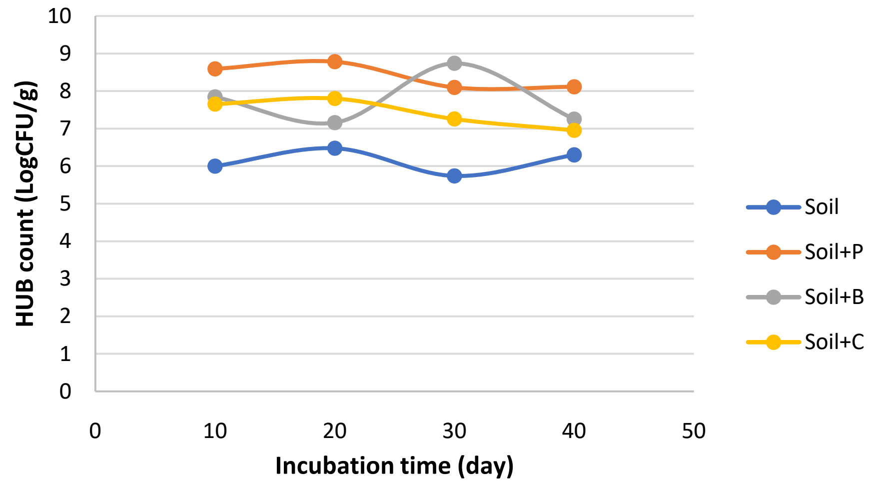

Figure 2. HUB loads in soils amended or not and supplemented with crude oil. Legend: P: Poultry manure, B: biochar-compost, C: cow dung.

according to Douglas *et al.* [24], higher counts in HUB might as well be due to the abilities of the organic wastes to neutralize the toxic effect of the oil on the microbial population by rapidly improving the physicochemical characteristics of the soil. In addition, organic amendments improved bacterial count in rhizosphere which resulted in higher removal of hydrocarbons [27]. Similarly, the amendments immobilized hydrocarbons via sorption, which alleviated hydrocarbon toxicity, thereby enhancing microbial growth [30] [31]. The high HUB load in the amended soils could also be explained by a contribution of HUB from the substrates used for the amendment. Considering the pH of the soil studied, we could also think that the increase in pH following the amendment of the soil could be the cause of stimulation of microbial growth in general and therefore that of HUB. During this study, an irregularity in the evolution of the HUBs was observed in both cases (diesel and crude oil) (Figure 1 and Figure 2).

Thus, HUB loads often decrease during experimentation. This reduction can be explained by a possible toxicity due to the contribution of hydrocarbons in non-amended soil [3]. In cases of amended soil, the reduction in the HUB load may due to the temporary toxicity of the media as a result of over-dose of the nutrient from the organic amendments [32].

3.3. Diesel and Crude Oil Biodegradation

In the case of diesel, the residual quantities decrease over time in amended or unamended soils (**Figure 3**). This observation is confirmed by the analysis of variance which shows that there is a significant difference between the residual diesel levels of days 10 and 20 and those of days 30 and 40 ($p < 0.0001$). The results show that in general, biodegradation is greater in amended soils (especially with biochar-compost). This observation is also confirmed by the analysis of variance which shows that the amendment has a significant effect on the biodegradation of diesel ($p = 0.048$).

In the case of crude oil, biodegradation seems to be greater than that observed at the diesel level from the 10th day. However, around the 40th day, the residual quantities of crude oil are relatively greater than those of diesel (**Figure 4**). The analysis of variance shows that in this case also, there is a significant difference between the residual quantities of crude oil on the 10th and 40th days ($p = 0.001$). It also shows that the amendment has a significant effect on the biodegradation of crude oil ($p < 0.0001$).

For both types of substrates (diesel and crude oil), the residual quantities decrease over time and are always lower in amended soils which means that the biodegradation rates of diesel and crude oil increase with time and amendment.

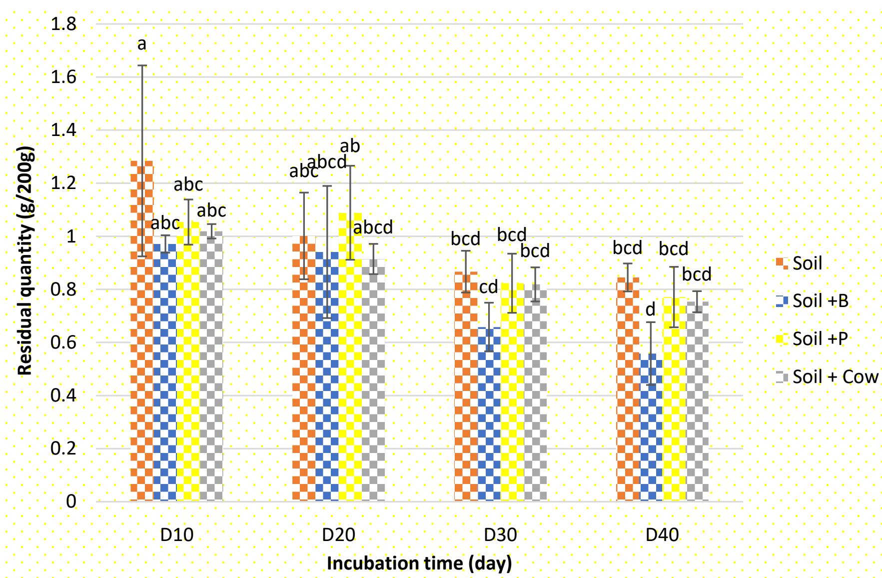

Figure 3. Residual quantities of diesel in soils amended or not with regard to incubation time. For all times and for all the substrates experienced, values sharing the same letter are not significantly different according to the Newman-Keuls' test at $p = 0.05$. Legend: P: Poultry manure, B: biochar-compost, C: cow dung.

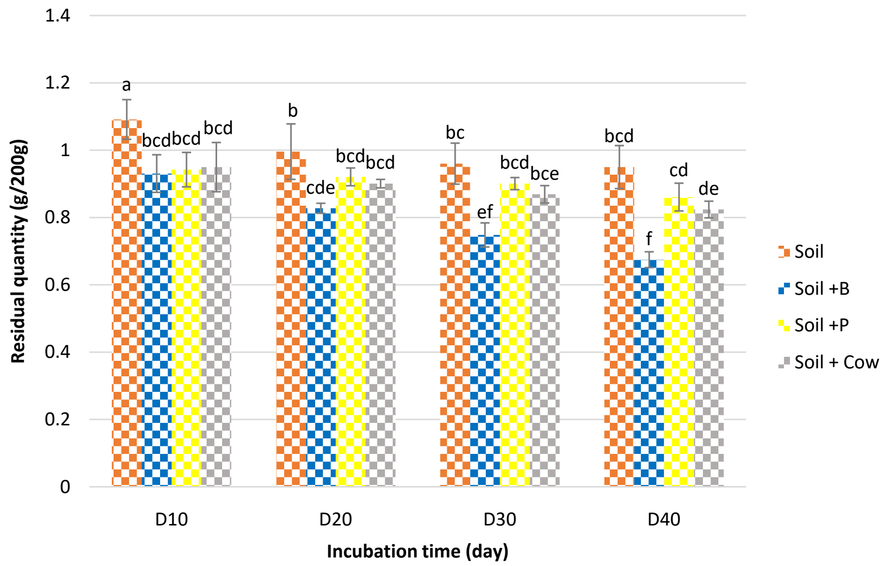

Figure 4. Residual quantities of crude oil in soils amended or not with regard to incubation time. For all times and for all the substrates experienced, values sharing the same letter are not significantly different according to the Newman-Keuls' test at p = 0.05. Legend: P: Poultry manure, B: biochar-compost, C: cow dung.

This finding is similar to that of several authors. Indeed, according to Agamuthu *et al.* [33], the addition of sewage sludge and cow dung in an used lubricant oil-contaminated soil (10%, w/w) resulted in 82% and 94% biodegradation, respectively, which were significantly higher than that in the control (without any amendment) setup (56%). In addition, Orji *et al.* [34], using cow dung as organic nutrient source has shown good promises in the bioremediation of crude oil impacted mangrove swamps in Nigeria. Similarly, Udume *et al.* [35], have revealed that the greatest biodegradation (93%) occurring in sterilized soil inoculated with optimized water hyacinth compost while that of the control was only 4% during crude oil biodegradation after 56 days incubation period. For Douglas *et al.* [24], Goat manure amended set-ups showed 23% biodegradation while the control setup gave only (16%) during soil hydrocarbons degradation. According to Koshlaf *et al.* [36], the addition of plant residues led to enhanced degradation (66.6%) at the beginning of the treatment during PAH degradation in soil.

The improvement in hydrocarbon biodegradation in amended soils can be explained by several reasons. So, according to Cai *et al.* [37], organic amendments can redistribute hydrocarbons from solid to aqueous phases by influencing the sorptive sites in soil particles, consequently enhancing hydrocarbons availability for microbial degradation. In addition, input of organic material resulted in enhanced nutrient retention in hydrocarbons contaminated soils, thereby affecting the growth of microbes and then improve hydrocarbons degradation [38] [39]. According to Mushtaq *et al.* [40], the organic amendment helps to mineralize resistant materials and eliminate xenobiotic compounds by promoting biotic and abiotic processes that rely on biodegradation and adsorption mechanisms. In addition, cow dung amended-soil was found to have improved soil physiochemical characteristics that enabled speedy adaptation by the microbes to the contaminate. These organic matters proved to enhance the multiplication of indigenous microbes thus enabling rapid biodegradation of the contaminant in the soil [24].

At the end of the experiments, the residual quantities of diesel are less significant than those of crude oil, which means that the biodegradation of diesel is greater than that of crude oil under our experimental conditions. The compositions of these substrates could explain this. Indeed, diesel contains more short-chain hydrocarbons, while crude oils mainly include long-chain hydrocarbons that are more difficult to degrade and show low solubility in water [41]. However, the analyze of variance shows that there is no significant difference between the residual quantities of diesel and crude oil ($p = 0.986$).

4. Conclusion

This study showed that biochar-compost, poultry manure and cow dung can be used to improve the biodegradation of diesel and crude oil in the studied soil. However, the increase in biodegradability after amendment is relatively low compared to those revealed by other studies. This means that it is necessary to evaluate the compositions of organic wastes in order to propose better formulations to further increase the biodegradation rates of diesel and crude oil in this type of soil.

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Conflicts of Interest

The authors declare that there is no conflict of interest related to this article.

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