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Bioremediation of Crude Oil Contaminated Soil Using Pig Droppings and Bone Char

Ejikeme Ugwoha[1], Victor Emeka Amah[1*] and Gabriel Obiosabofu Oweh[1]

[1]Department of Civil and Environmental Engineering, University of Port Harcourt, P.M.B. 5323, Rivers State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author VEA designed the study, performed the statistical analysis and wrote the protocol. Author EU wrote the first draft of the manuscript. Authors EU, VEA and GOO managed the analyses of the study. Author GOO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Oil extraction operations as well as equipment failure and infrastructure vandalism have caused serious environmental pollution with crude oil spills world-wide. The remediation of the polluted sites is an environmental problem beckoning for solution. In this study, the possibility of pig droppings and pig bone char mixture (biostimulant) to stimulate and optimize crude oil biodegradation in soil was investigated. Exactly 500g of loamy soil was spiked with 3% (w/w) of crude oil. The spiked soil was amended with varying percentage mixtures of the biostimulant and labelled A – E. The spiked soil without biostimulant served as the Control. Each experiment was setup in six (6) replicates, carried out for six weeks, and destructively sampled and analysed on a weekly basis. The removal efficiencies of the biostimulated and unbiostimulated soils were observed to range from 66.70 to 86.70% and 3.69%, respectively. The biodegradation first-order rate constants ranged from 0.1978 to 0.3391wk^{-1} and 0.0050wk^{-1} for the biostimulated and unbiostimulated soils, respectively. Optimum removal of total petroleum hydrocarbon (TPH) was observed for biostimulated soil C comprising 50% bone char and 50% pig droppings. Results from biostimulated soils A, B, D and E indicated that pig droppings is a more effective biostimulant than pig bone char. A first-order kinetic model adequately predicted the removal of TPH with the optimum biostimulant. It is concluded that using

**Corresponding author: E-mail: victor.amah@uniport.edu.ng;*

agro-organic waste materials such as pig droppings and pig bone char in a ratio of 1:1 can offer a simple, effective, inexpensive and environmentally friendly solution to the problem of soil contamination with crude oil.

Keywords: Bioremediation; crude oil; pig droppings; bone char; contaminated soil.

1. INTRODUCTION

Soil pollution with crude oil and its products has become a major global environmental concern. Crude oil spill arises from vandalism of oil installations, corrosion of over aged oil facilities and uncontrolled spillage in oil refineries and storage tanks [1,2,3]. Crude oil is a complex mixture containing thousands of hydrocarbons [1,2] that can be divided into four classes, namely the saturates, the aromatics, the asphaltenes and the resins [4]. It is physically, chemically and biologically harmful to soil because it contains many toxic compounds in relatively high concentrations [1], and is thus classified as environmental pollutant by the US Environmental Protection Agency [5,4]. When crude oil is released on the ground surface, it gradually penetrates the soil and at a depth of around 10-20 cm, it results in soil fertility loss. Other effects are environmental degradation, groundwater pollution, biodiversity loss and threat to environmental sustainability [2,3,5]. Under normal conditions, crude oil in soil persists much longer than most conventional carbon sources (e.g. carbohydrate and proteins) which take only weeks to be degraded, while under extreme conditions (e.g. drought) it persists much longer [6].

Remediation of petroleum hydrocarbons contaminated sites is a real-world problem [7]. Over the years, several methods have been developed and investigated for the remediation of petroleum hydrocarbons contaminated sites. Some of the major methods are physicochemical, thermal and biological techniques [1,4]. The choice of the method to use depends on the chemical, physical and biological properties of both contaminant and soil [1]. The physicochemical and thermal techniques have been found to be expensive and labourious [6]. Bioremediation (biological technique) has appeared as the most desirable method due to its simplicity, cost-effectiveness and eco-friendliness [2,3,5,4,8]. Bioremediation is a treatment process that uses microorganisms to breakdown or degrades hazardous substances into less toxic or nontoxic substances [1]. Critical conditions for effective bioremediation include the presence of contaminants, microbes that feed on the contaminants, sufficient oxygen, suitable soil moisture, right temperature, nutrients to support microbe growth, and suitable pH [1,9].

Naturally, bioremediation can be slow due to the presence of high molecular weight compounds with very low solubility [1]. More so, the oxidizing microorganisms may not be present in contaminated soil in the numbers required for effective bioremediation [2]. In order to improve the natural tendency of soil microorganisms to decompose hydrocarbons from crude oil, many techniques have been proposed and tested. These techniques include the use of amendments [1,10,5] and microorganism immobilization [11]. Accordingly, bioremediation could be achieved either as biostimulation (addition of nutrients/amendments) or bioaugumentation (addition of oxidizing microorganisms), depending on the pollution situation and type of microorganisms being used [8]. But biostimulation has been proven to be a promising bioremediation technique for the treatment of polluted soil aerobically [3].

Nigeria is blessed with agricultural wastes and by-products (e.g. dungs and bones) which are considered useless to the ordinary man, but has been shown to be useful materials to modify the soil physical and chemical properties as well as release nutrients for microbial activities [3]. The accumulation of agricultural wastes and by-products without proper programmes for collection, transportation and disposal constitute a major environmental problem. More so, the use of pig droppings and bones to increase biodegradation of contaminated soil adds value to the management of slaughter waste and decreases the biochemical oxygen demand loads on rivers and streams around slaughterhouses. Therefore, using such materials as amendments in the bioremediation of crude oil contaminated soil becomes thoughtful. Accordingly, several organic amendments have been used by researchers, namely cow dung and inorganic fertilizer, cow dung and palm kernel husk ash [3], bone char from cow bones [5] and goat droppings [12]. To

the best of our knowledge, the use of pig droppings and bone char amendment to enhance the bioremediation of crude oil contaminated soil has not been reported. Thus, this research is aimed at understanding the bioremediation level of soil contaminated with crude oil using organic stimulants (pig droppings and bone char) capable of delivering nutrients in order to enhance microbial degradation.

2. MATERIALS AND METHODS

2.1 Crude Oil

The crude oil used in this study was obtained from Obonagha Flow Station in Yenegoa Local Government Area of Bayelsa State. The crude oil was analyzed for total petroleum hydrocarbon (TPH) to obtain a baseline value.

2.2 Soil

The soil sample used was uncontaminated loamy soil obtained from Obonagha Community in Yenegoa Local Government Area of Bayelsa State. The soil was dug from a depth of 0 – 15 cm using a standard auger. The soil was analysed for pH, total organic carbon, organic matter, porosity and moisture content using standard methods, to establish baseline condition before use in the experiment. [13] Identified three hydrocarbon degrading bacteria generally present in soil samples taken from Bayelsa state namely: Pseudomonas aeroginosa, Staphylococcus aureus and Lactobacillus acidophilus. The occurrence of Pseudomonas spp., Staphylococcus spp. and Lactobacillus spp. indicates that these bacterial genera are well established as hydrocarbon degraders. The characteristics of the soil sample are summarized in Table 1.

Table 1. Characteristics of loamy soil sample

Parameters	Method	Value
pH	APHA 4500–H^+B	5.81
Total organic carbon (%)	ASTMD 2974	1.981
Organic matter (%)	ASTM D4129	7.0
Nitrate (NO_3) (mg/20g)	APHA 4500 NO_3E	6.47
Electrical conductivity (millisiemens/cm)	APHA 2510B	475
Phosphate (PO_4) (mg/kg)	APHA 4500P.E	0.071
Porosity (%)	ASTM C830	42.3
Moisture content (%)	ASTM D2216	9.94

2.3 Pig Droppings and Bone Char

Pig droppings were obtained from a Pig farm in Omagwa in Obio/Akpor Local Government Area of River State. The pig droppings were sun-dried for two weeks, grinded using mortar and pestle and sieved using a 2mm standard mesh sieve (Fig. 1). Pig bones were obtained from Trans Amadi slaughter in Obio/Akpor Local Government Area of River State. The pig bones were sun-dried for two days, charred at a temperature of 500^oC for 45 minutes in a muffle furnace, grinded using mortar and pestle and sieved using a 2mm standard mesh sieve (Fig. 1). The characteristics of the pig droppings and bone char samples are summarized in Table 2.

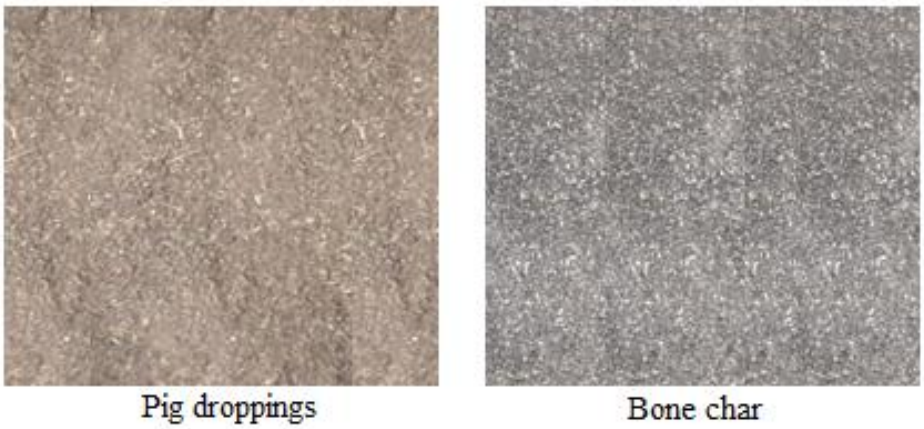

Fig. 1. Pig droppings and bone char samples

Table 2. Pig droppings and bone char characteristics

Parameters	Method	Pig droppings	Bone char
pH	APHA 4500 $-H^+B$	7.60	8.00
Total organic carbon (%)	ASTMD 2974	<0.01	3.93
Organic matter (%)	ASTM D4129	12.0	21.5
Nitrate (NO_3) (mg/20g)	APHA 4500 NO_3E	25	2.44
Electrical conductivity (EC)	APHA 2510B	2710	1337
Phosphate (PO_4) (mg/kg)	APHA 4500P.E	0.307	0.490

2.4 Design of Experiment

The experiment was designed as shown in Table 3. Each experiment was done in six (6) replicates because the experiment duration was six (6) weeks and a destructive sampling method was employed.

2.5 Experimental Setup

The experiment was carried out in a perforated 1.5-litre plastic container with an estimated depth of 13 cm following the procedure by [3] (Fig. 2). In the 1.5-litre plastic perforated container, 500 g of the loamy soil was placed and mixed thoroughly with 15 g (3% w/w) of crude oil. Pig droppings and bone char were added to the contaminated soil in different mix ratio as described in Table 3. The setup was thoroughly mixed and watered with 4 ml of distilled water every two (2) days to keep the moisture content constant. The setup was monitored for six (6) weeks. Sampling was destructively performed on a weekly basis.

Table 3. Design of experiment

Treatment	Biostimulants (g)	Biostimulants (%)
Control	0 BC + 0 PD	0 BC + 0 PD
A	100 BC + 0 PD	100 BC + 0 PD
B	80 BC + 20 PD	80 BC + 20 PD
C	50 BC + 50 PD	50 BC + 50 PD
D	20 BC + 80 PD	20 BC + 80 PD
E	0 BC + 100 PD	0 BC + 100 PD

BC=Bone Char; PD=Pig Droppings

Fig. 2. Experimental setup

2.6 Soil Sampling and Analysis

Ten grams (10 g) of soil sample was destructively taking from each setup on a weekly basis. Thirty milliliters (30 ml) of dichloromethane (DCM) was added to the soil sample and agitated using mechanical shaker for 30min. Before the extraction, 50ppm of a surrogate standard (o-terphnyl) was spiked to the soil sample to measure the percentage recovery of the extraction method. The extract was filtered using a glass wool and was kept in a fume closet to concentrate to 1ml and the concentrate was transferred to a 2 ml Agilent vial for injection into an Agilent 7890 Gas Chromatography with Flame Ionization Detector (GC-FID) having a split/splitless inlet module and a capillary column (30 m * 0.32 mm * 0.25 µm). The GC-FID setting for TPH analysis was as described in Table 4.

2.7 Biodegradation Efficiency

The biodegradation efficiency, a measure of how much the contamination level has dropped over time due to the introduction of amendments, was computed using Equation (1) [3].

$$D = \frac{C_o - C_t}{C_o} \times 100\% \quad (1)$$

Where D is the biodegradation efficiency (%), C_o is the initial TPH in soil sample (mg/kg) and C_t is the residual TPH in soil sample at any time (mg/kg).

2.8 Biostimulation Efficiency (B.E)

The biostimulation efficiency, an insight to the treatability options available by the biostimulants, was calculated using Equation (2) [3].

$$B.E = \frac{D(T) - D(U)}{D(T)} \times 100\% \quad (2)$$

Where D(T) is the percentage removal of crude oil in the biostimulated soil and D(U) is the percentage removal of crude oil in the control soil.

2.9 Bioremediation Kinetics

Biodegradation rate of organic compounds by microorganisms is often described by first-order kinetic equation shown as Equation (3) [3].

$$\ln C_t = \ln C_o - kt \quad (3)$$

Where C_o is the initial TPH in soil sample (mg/kg), C_t is the residual TPH in soil sample at any time (mg/kg), *k* is the biodegradation rate constant ($week^{-1}$) and t is the time (week). The ability of the model to describe the biodegradation process was evaluated using coefficient of determination (R^2) whose value lies between 0 and 1, where values close to 0 indicate poor model fit and values close to 1 indicate good model fit.

2.10 First-Order Half-Life

The first-order half-life ($t_{1/2}$), the time required for the contaminant concentration to reduce to half of its original concentration, was calculated using Equation (4).

$$t_{1/2} = \frac{\ln 2}{k} \quad (4)$$

Where *k* is the biodegradation rate constant.

Table 4. GC-FID condition for TPH analysis

Inlet condition	Oven condition	Detector condition	Column
Mode: Constant pressure (Split mode) Initial temp: 300°C Pressure: 30.0 psi Split ratio: 10:1 Split flow: 12.0 ml/min Total flow: 15.8 ml/min Gas saver: Off Gas type: Helium	Initial temp: 40 °C(On) Initial hold time: 1.00 Equilibration time: 0.10 Ramp: 10 °C/min Final temp: 320 °C Final hold time: 11 min Run time: 40 min	Temp: 300°C H_2 flow: 30 ml/min Air flow: 400 ml/min Makeup flow (He): 25 ml/min	Type: Agilent DB5 Length: 30 m Diameter: 0.32 mm Film thickness: 0.25 µm

3. RESULTS AND DISCUSSION

3.1 Biodegradation of Total Petroleum Hydrocarbon (TPH)

Figs. 3 and 4 present the biodegradation rate and the percentage removal respectively of TPH for different soil treatments. Two-way analysis of variance was done to determine any significant change in TPH concentration with time and across samples shown as Table 5. The P-values obtained are below 0.05, which indicates that there is significant change in TPH concentration across the different samples and with time. The degradation and percentage removal of TPH in the Control sample was negligible compared to those of the treated samples. The Control sample decreased only from 2302 to 2217 at the end of the six-week period of the experiment, representing only 3.69% TPH removal. On the contrary, the treated samples decreased from 2297 to 765 for treated sample A, from 2290 to 631 for treated sample B, from 2285 to 317 for treated sample C, from 2294 to 561 for treated sample D, and from 2270 to 512 for treated sample E, representing 66.70, 72.45, 86.70, 75.54 and 77.44% TPH removal, respectively. Samples D and E showed such high TPH removal because, the pig droppings contains higher Nitrate concentration when compared with the Bone char. The optimum TPH degradation was observed in treated sample C with 50% BC and 50% PD. The optimum TPH degradation observed in treated sample C compared to the other treated samples (A, B, D and E) could be linked to synergetic effect of equal proportion of bone char and pig droppings. The BC and PD complemented each other in adding essential nutrients such as Nitrates and Phosphate. Tables 1 and 2 show that PD has more Nitrates and both PD and BC has more Phosphate than the native soil. This improved the nutrients in the native soil sample which was crucial to the growth of the existing hydrocarbon degrading microorganisms. Similar range of percentage TPH removal (74 – 85%) was reported by [5] with cow bone char. [3] achieved 84.62, 71.80 and 58.60% TPH removal with cow dung and inorganic fertilizer (NPK) and 64.44, 58.60 and 45.31% TPH removal with cow dung and palm kernel husk ash, at 2, 4 and 6% (w/w) crude oil spill, respectively. [12] achieved 70.5 and 92.6% TPH removal with 10 and 15% by weight of goat droppings, respectively. The very poor level of degradation in the Control sample (0% BC and 0% PD) could be attributed to lack of required nutrients, leading to poor growth of the hydrocarbon degrading microorganisms needed to cause rapid decline in TPH levels as seen in the biostimulated soil samples. Overall, the result indicates that the mixture of bone char and pig droppings in the ratio of 1:1 could make an effective biostimulant to enhance the bioremediation of crude oil contaminated soil.

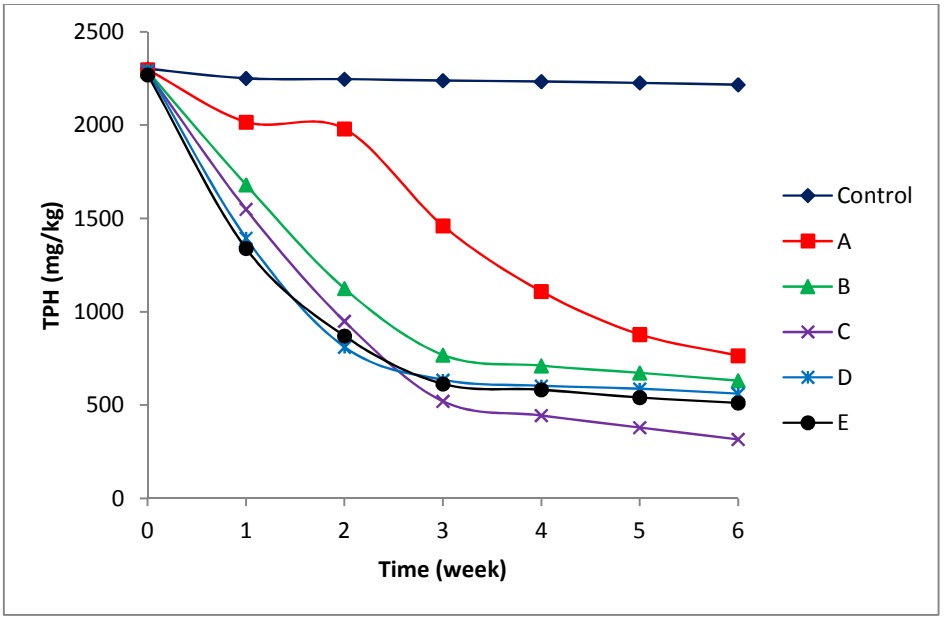

Fig. 3. Biodegradation rate of TPH for different soil treatments

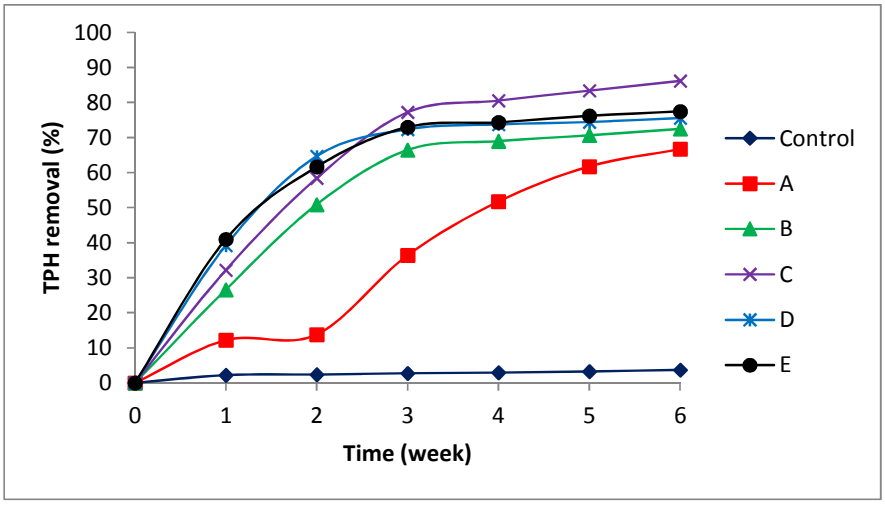

Fig. 4. Percentage removal of TPH for different soil treatments

Table 5. Analysis of variance showing differences in time and samples

Source of Variation	SS	df	MS	F	P-value	F crit
Time (weeks)	10373328	6	1728888	20.08	2.81E-09	2.42
Samples	9253114	5	1850623	21.49	4.24E-09	2.53
Error	2583226	30	86107.54			
Total	22209668	41				

SS = Sum of squares; df = Degrees of freedom; MS = Mean squares; F = F-statistic

3.2 Biostimulation Efficiency

Biostimulation efficiency (B.E) could be explained as a measure of the impact of adding biostimulant to enhance the biodegradation process with respect to a Control setup where no biostimulant was introduced. The B.E in this study was computed using Equation (2) and presented in Fig. 5. At the end of the six-week experimental period, B.E for treated samples A, B, C, D and E was found to be 94.47, 94.91, 95.74, 95.12 and 95.24%, respectively. Treated sample C which is a combination of 50% BC + 50% PD has the best performance of 95.74% B.E. Treated samples D and E having higher B.E than treated samples A and B with the same but swapped percentage mixture of biostimulants, indicates that pig droppings is a more effective biostimulant than pig bone char. [3] achieved B.E of 62.1, 58.1 and 51.7% with cow dung and inorganic fertilizer (NPK) and 50.2, 48.7 and 37.5% with cow dung and palm kernel husk ash, at 2, 4 and 6% (w/w) crude oil spill, respectively. The present study used 3% (w/w) crude oil spill and had better B.E than [3] 2 – 6% (w/w), indicating that pig droppings and bone char mixture is a better biostimulant than cow dung and inorganic fertilizer (NPK) mixture and cow dung and palm kernel husk ash mixture for the enhancement of bioremediation of crude oil contaminated soil.

3.3 Kinetics of Biodegradation of Crude Oil in Soil

Figs. 6 to 11 present linear plots obtained from linear regression and from these Figures coefficient of determination (R^2) values in Table 6 were generated. The linear plots show the first-order kinetic rate constant for each treatment. The high values of the coefficient of determination ($R^2 > 0.7$) imply the attainment of a good first-order kinetic rate constant for the biostimulants. The slope of the plot is the first-order kinetic constant (k) of Equation (3). From the plot, it can be observed that the Control sample has an almost horizontal slope, indicating a very low degradation of crude oil in the soil with time. Among the treated samples, the values of the reaction rate constant show that the biostimulant in treated sample C enhanced the degradation of crude oil in the soil more efficiently than the biostimulants in treated samples A, B, D and E. The values of reaction rate constant were substituted into Equation (3) to obtain the first-order kinetic models in Table 6.

The crude oil biodegradation half-life for each treatment was computed using Equation (4). The kinetic parameters of the first-order degradation models (Table 6) show that the highest rate of crude oil degradation occurred in treated sample C (k = 0.3391 week^{-1}) with 86.70% removal efficiency and half-life of 2.04 weeks while the least occurred in Control sample (k = 0.0050 week^{-1}) with 3.69% removal efficiency and half-life of 138.63 weeks.

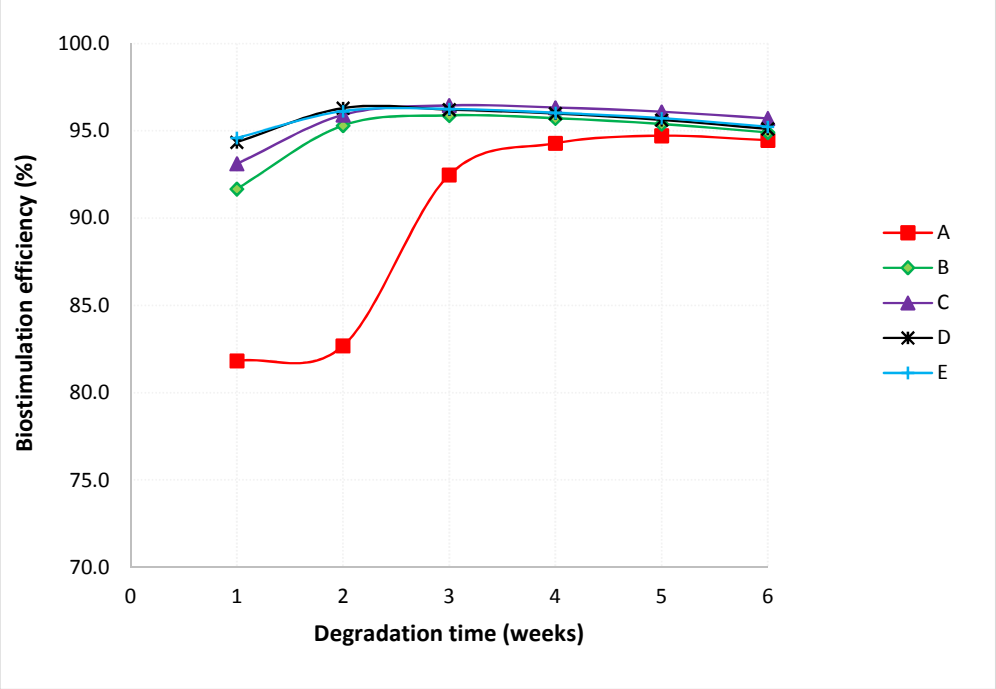

Fig. 5. Biostimulation efficiency of different biostimulant mixtures

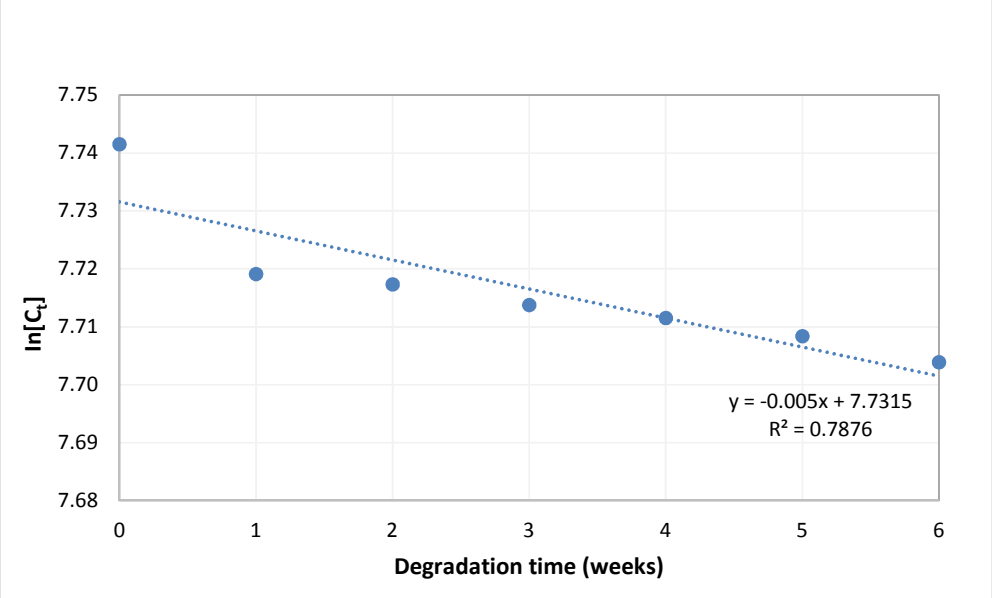

Fig. 6. First-order kinetic rate constant determination for control sample

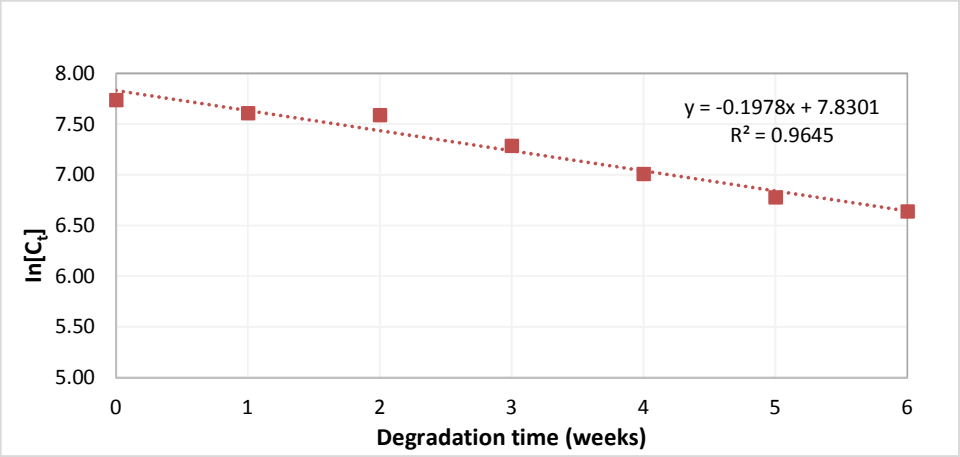

Fig. 7. First-order kinetic rate constant determination for sample A

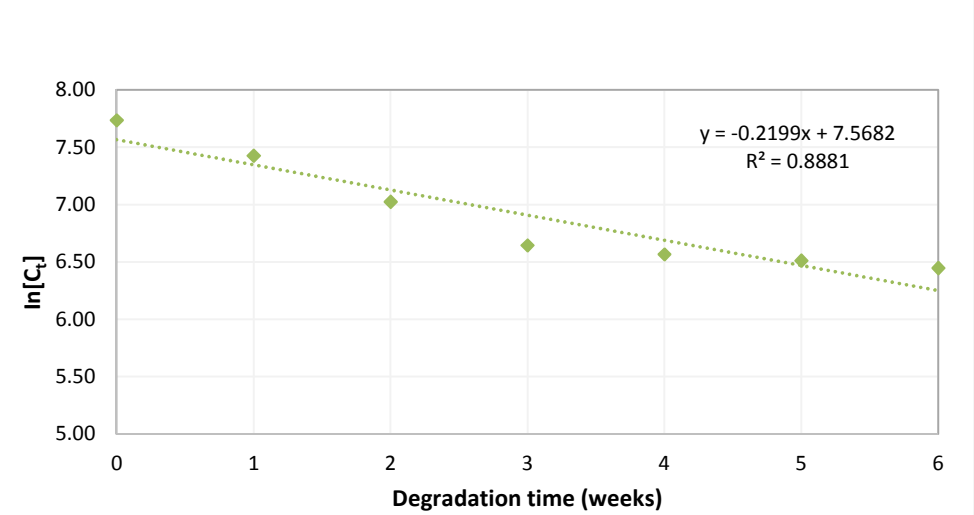

Fig. 8. First-order kinetic rate constant determination for sample B

Table 6. Biodegradation prediction models, half-life and kinetics parameters for crude oil in soil with bone char and pig droppings mixture treatment

Treatment	Biostimulants	1st-order kinetic equation	1st-order rate constant, *k* (week^{-1})	$t_{1/2}$ (weeks)	R^2
Control	0% BC + 0% PD	$\ln[C_t] = \ln[C_o] - 0.0050(t)$	0.0050	138.63	0.7876
A	100% BC + 0% PD	$\ln[C_t] = \ln[C_o] - 0.1978(t)$	0.1978	3.50	0.9645
B	80% BC + 20% PD	$\ln[Ct] = \ln[C_o] - 0.2199(t)$	0.2199	3.15	0.8881
C	50% BC + 50% PD	$\ln[Ct] = \ln[C_o] - 0.3391(t)$	0.3391	2.04	0.9429
D	20% BC + 80% PD	$\ln[Ct] = \ln[C_o] - 0.2233(t)$	0.2233	3.10	0.7997
E	0% BC + 100% PD	$\ln[Ct] = \ln[C_o] - 0.2387(t)$	0.2387	2.90	0.8502

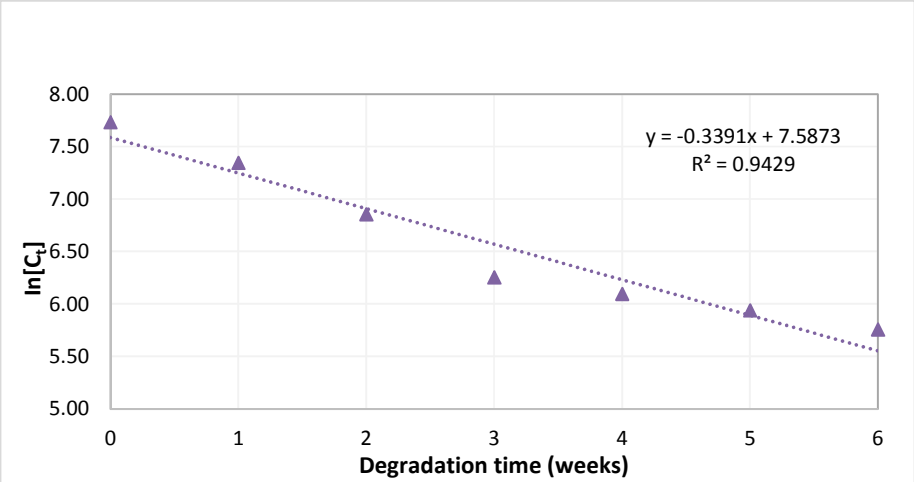

Fig. 9. First-order kinetic rate constant determination for sample C

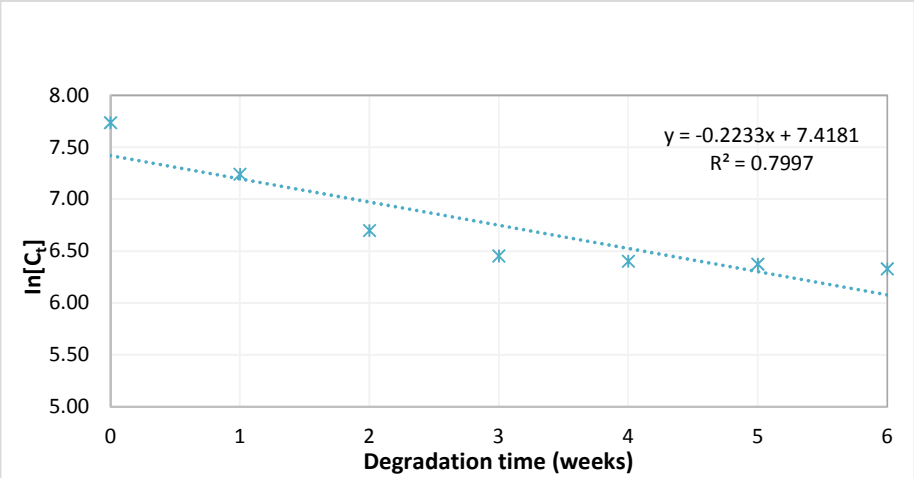

Fig. 10. First-order kinetic rate constant determination for sample D

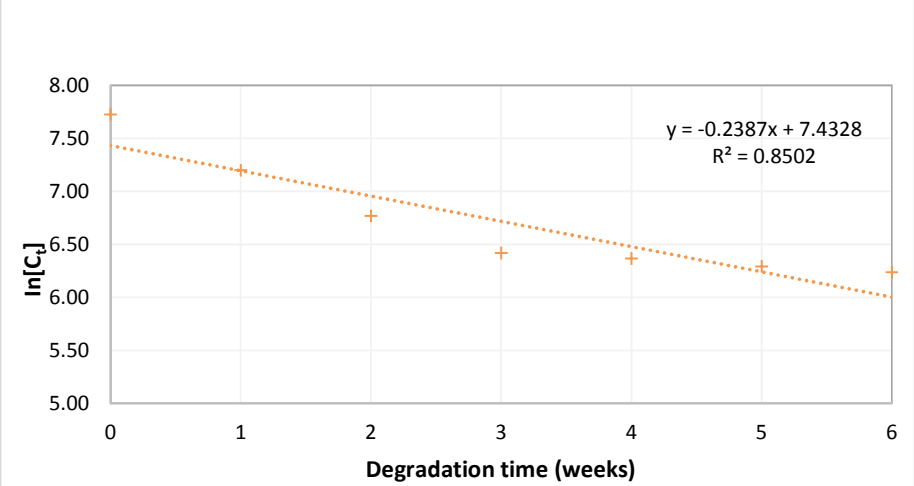

Fig. 11. First-order kinetic rate constant determination for sample E

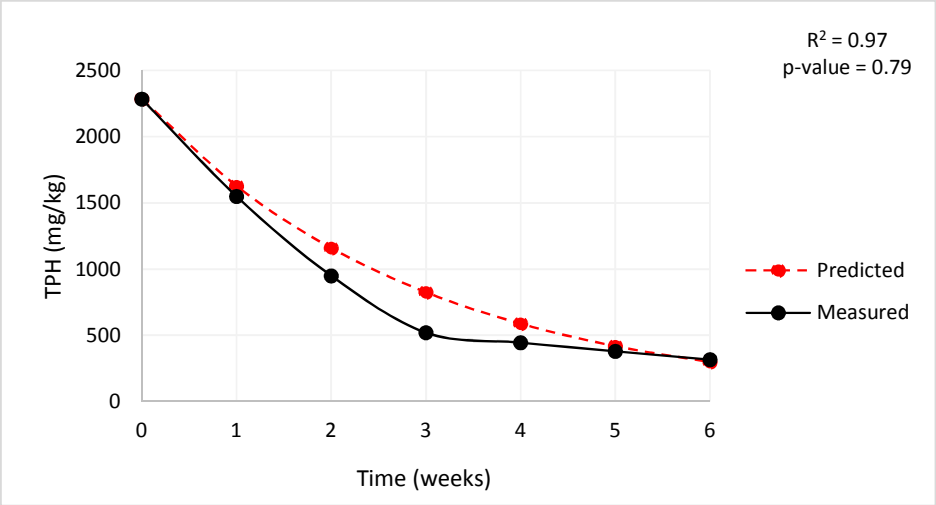

Fig. 12. Measured and predicted biodegradation of crude oil in soil treated with 50% bone char and 50% pig droppings mixture

3.4 Model Validation

The validation of the biodegradation model for the optimum biostimulant (treated sample C) using the relationship between the measured and predicted TPH concentrations is presented in Fig. 12. The high coefficient of determinations (R^2 = 0.97) obtained indicate good agreement between the measured and predicted biodegradation of crude oil in soil. More so, P = 0.79 was obtained, which is greater than the Alpha value (0.05), implying that there is no significant difference between the measured and predicted crude oil biodegradation.

4. CONCLUSION

The application of biostimulant (pig droppings and bone char mixture) to enhance the bioremediation of crude oil contaminated soil for six weeks revealed a substantial increase in the degradation of crude oil in the soil. Among the different percentage mixtures, 50% pig droppings and 50% bone char gave the optimum enhancement of crude oil degradation. The performance of the other percentage mixtures suggested that pig droppings is a more effective biostimulant than pig bone char. Finally, a first order kinetic model could be employed in fitting TPH degradation from crude oil contaminated soil following amendments with pig droppings and pig bone char. Thus, using agro-organic waste materials such as pig droppings and pig bone char in a ratio of 1:1 can offer a simple, effective, inexpensive and environmentally friendly solution to the problem of soil contamination with crude oil.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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