Bio-stimulation with Fresh Avocado Seed for crude oil Remediation with a Bio-kinetic Modelling

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Direct Research Journal of Engineering and Information Technology

Vol. 14(2), Pp.137-142, 2026,

https://journals.directresearchpublisher.org/index.php/drjeit; https://www.ajol.info/index.php/drjeit

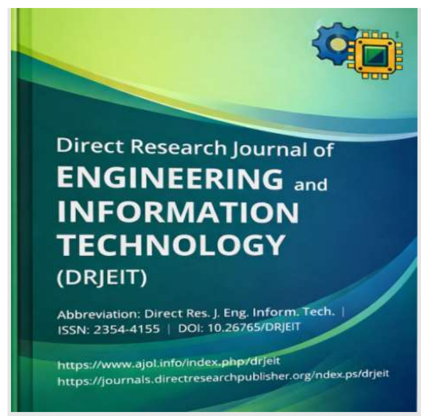
Research Article
ISSN: 2354-4155

Received 8 March 2026, Accepted 23 May 2026, Published 30 July 2026

ABSTRACT

The remediation of crude oil-contaminated soils requires sustainable and cost-effective solutions. This study investigates the performance of fresh avocado seed (Persea americana) as a biostimulant for the remediation of Bonny Light crude oil-contaminated soil from the Kalarugbana community, Niger Delta. A 56-day laboratory-scale experiment was conducted using bioreactors containing 1 kg of soil contaminated with 100 ml of crude oil and treated with different volumes of fresh avocado seed slurry (0 ml as control, 100 ml, 200 ml, and 300 ml). Microbial growth (CFU/g) and Total Petroleum Hydrocarbon (TPH) depletion (mg/kg) were monitored weekly. Results demonstrated a strong positive correlation between inoculants volume and efficacy. Microbial counts increased from 5,000 CFU/g to 114,000, 135,000, and 170,000 CFU/g for the 100 ml, 200 ml, and 300 ml treatments, respectively. This enhancement directly drove TPH depletion from an initial 3,729.7 mg/kg to 1,202.4, 1,038.1, and 647.7 mg/kg, corresponding to degradation efficiencies of 67.76%, 72.17%, and 75.33%. The control reactor showed only minimal natural attenuation (38.3%). A biokinetic model was developed from first principles, relating substrate depletion to remediator concentration. Model simulations in MATLAB, using an equilibrium rate constant (K-eq) of 0.0083 week^{-1}, produced results in strong agreement with the experimental data. This work confirms that fresh avocado seed is a highly effective, low-cost biostimulant, with its efficiency directly scalable to the volume applied. The validated model provides a predictive tool for optimizing remediation strategies.

Keywords: *Biostimulation, Avocado Seed, Phytoremediation, Total Petroleum Hydrocarbon (TPH), Biokinetic Model, Niger Delta.*



Citation: Eugene, M.N. & Sunday-Piaro, T. (2026). Bio-stimulation with Fresh Avocado Seed for crude oil Remediation with a Bio-kinetic Modelling. *Direct Research Journal of Biology and Biotechnology*. Vol. 12(1), Pp. 137-142. https://dx.doi.org/10.4314/drjeit.v14i2.2

INTRODUCTION

Niger Delta region of Nigeria, a hub of crude oil exploration, faces severe environmental degradation due to frequent oil spills (Ite etal, 2013). These incidents lead to the contamination of agricultural lands, reducing soil fertility and posing significant health risks (Figure 1) (Lim et al, 2016). Conventional remediation methods like chemical and thermal treatment are often expensive and can be environmentally disruptive (Isitekaleaka). Consequently, there is a growing emphasis on bioremediation techniques, which leverage biological processes to degrade pollutants (Olatunji et al, 2013). Biostimulation, a subset of bioremediation, involves the addition of nutrients to enhance the growth and activity of indigenous hydrocarbon-degrading microorganisms (Mary et al 2009). While various biostimulants have been explored, the search for cost-effective and readily available materials remains crucial, especially in resource-limited settings. The avocado seed (Persea

Figure 1: Crude oil Spilled on Eleme Community in Rivers State.Crude oil Spilled on Eleme Community in Rivers State.

Figure 2: Avocado (Persea Americana) Seed Fruits, Leaves and Seed Avocado (Persea Americana) Seed Fruits, Leaves and Seed

americana), a common agricultural waste product, is rich in carbohydrates, proteins, lipids, and essential minerals like phosphorus, potassium, and iron (Figure 2). These components are known to support microbial metabolism, suggesting its potential as a effective biostimulant (Oluremi et al, 2009). Although preliminary studies have indicated the remedial potential of avocado composites [8, 9], a significant research gap exists in the quantitative modelling of its performance. Predictive models are essential for optimizing application rates, estimating remediation timelines, and scaling up laboratory findings to field applications. This study, therefore, aims to:

1. Experimentally evaluate the efficacy of fresh avocado seed as a bio-stimulant for crude oil-contaminated soil.
2. Develop and validate a biokinetic model to predict the degradation kinetics of Total Petroleum Hydrocarbons (TPH) based on remediator concentration.

MATERIALS AND METHODS

Sample Collection and Preparation

Soil and Bonny Light crude oil samples were collected from Kalarugbana community, Okrika, Rivers State, Nigeria. Fresh avocado seeds were obtained, washed, and ground into a fine slurry using a mechanical grinder. The physicochemical properties of the soil, crude oil, and avocado seed were characterized using standard analytical methods (APHA, ASTM).

Experimental Design and Setup

The experiment was conducted over 56 days (8 weeks) in a batch reactor system. Six plastic bioreactors were set up, each containing 1 kg of soil. Each reactor was contaminated with 100 ml of Bonny Light crude oil and homogenized. The experimental design was as follows:
Control (CT): Contaminated soil + 0 ml avocado slurry.
CT100: Contaminated soil + 100 ml avocado slurry.
CT200: Contaminated soil + 200 ml avocado slurry.
CT300: Contaminated soil + 300 ml avocado slurry.

The reactors were kept under aerobic conditions. Soil moisture was maintained at optimal levels by weekly addition of 100 ml of distilled water. The contents were mixed twice a week to ensure aeration and homogeneity.

Analytical Methods.

Microbial Analysis: Total bacterial count (TBC) was determined weekly using the standard pour plate method on nutrient agar, incubated at 30°C for 48 hours. Results were expressed as Colony Forming Units per gram of soil (CFU/g). Total Petroleum Hydrocarbon (TPH) Analysis: TPH concentration was determined weekly using a Gas Chromatograph with a Flame Ionization Detector (GC-FID) following USEPA Method 1625 after solvent extraction. Percentage TPH Degradation: the degradation efficiency was calculated using Equation 1:

$$D = \left(\frac{TPH_i - TP_f}{TPH_i}\right) \times 100 \qquad (1)$$

where TPH_i is the initial TPH concentration and TPH_f is the final TPH concentration at time (t)

Biokinetic Model Development

A model was developed based on Michaelis-Menten enzyme kinetics, considering the avocado seed composites as catalytic agents (E) and the hydrocarbons as the substrate (S). The general reaction is expressed as

$$E_A + S \underset{K_2}{\overset{K_1}{\rightleftharpoons}} [ES] \underset{K_4}{\overset{K_3}{\rightleftharpoons}} P + E$$

From the material balance in a batch system the substrate depletion rate is given by:

$$\frac{dS}{dt} = \frac{X}{Y}\left[\frac{r_{max}\,S}{K_{eq}+S}\right] \qquad (2)$$

Where:
S = Substrate concentration(TPH,mg/kg)
X = Microbial cell density(CFU/g)
Y = Yield coefficient(mass cells/mass substrate)
r_{max} = Maximum specific growth rate (week^{-1})
K_{eq} = Substrate dissociation constant (week^{-}2)
Integrating Equation 2 under the experimental conditions yielded a predictive model for TPH concentration over time. The model was implesmented and simulated using MATLAB R2023a .

RESULTS AND DISCUSSION

Physicochemical Properties of the Kalarugbana community Soil

The Kalarugbana community soil being a river rain soil type is been identified from analytical assessment to be of a very high moisture content (Agari et al 2013), consisting of effective microorganisms which are indeed oxygen dependent in nature. The result from the soil analysis proves the soil to consist common salts liable to conductivity, with an electrical conductivity of 132μS/cm, with a very low nitrogen composite, and acidic in nature (Oluremi etal 2016). The soil generally as seen in (Table 1) proves to be poor in nutrient, such that the presence of hydrocarbon contaminants can drastically destroy the quality of the soil, such that even the small-scale agricultural production can be affected, as presently recoded in the area (Ukpala, 2017). The soil was identified to consist of some microorganisms which could be augmented with avocado seed or any phytoremediant for effective degradation of any active hydrocarbon contaminants over a defined period. These microorganisms are mainly of bacteria and fungi.

Physical Properties of the Bonny-Light Crude Oil

Presented in (Table 2), Are the outcomes derived from the physical characterization of the Bonny-light crude oil sample achieved experimentally. By employing gas chromatography-mass spectroscopy (GCMS) analysis for the Bonny-light crude oil (Raji and Hart, 2012), different mixtures of hydrocarbons with different component composition were revealed as seen grouped in

Table 1: Physicochemical Properties of Kalarugbana community Soil.

Parameter	Kalarugbana	Unit
Ph	5.46	-
E. C.	132	μS/cm
Total Phosphorus (%)	0.01	-
Total Nitrogen (%)	0.13	-
Total Organic Carbon (%)	0.281	-
Organic matter (%)	2.15	-
Moisture content (%)	9.91	-
Porosity (%)	36	-
Bulk Density	1.4174	g/cm^3

Table 2: Physical Properties of the Bonny-Light Crude Oil

Property	Result	Unit
Specific Gravity	0.8041	
Flash Point	75	°C
Basic Sediments and Water	0.453	%
Viscosity	14.372	Cp
Salt Content	4.3	mg/L
API	33.1	
Sulfur	0.12%	

Table 3: Physiochemical composition of fresh Avocado seed**.**

Properties	Amount
Protein	2.70g
Fat and oil	18.89g
Carbohydrate	65.76 g
Calories	186.8
Moisture content	16.67
pH	5.6

percentages based on their functional groups. Analysis of the avocado seed used in this work defines the seed to be of a high moisture content and rich in calories plus carbohydrates which are favorable to microbial growths for effective phytoremediation for a crude oil contaminated soil at time stamp. The analysis which was taken on a 100g fresh avocado seed results to the following composites as detailed in (Table 3). From the analysis it can be seen that 100g of fresh avocado seed consist of protein (2.70g), Fat (18.89g) and carbohydrate (65.76g) which are all favorable to microbial growth and performance. Vital 2001. Hence the concentration of the avocado seed is determined as the ratio of the amount of the active composts of the avocado seed (87.35g) by the volume of contaminated soil which is been added into for remediation process to take path.

Microbial Growth Dynamics

The impact of avocado seed addition on microbial population is shown in (Figure 3). A significant increase in microbial counts was observed in all treated samples compared to the control. The 300 ml treatment showed the most robust growth, reaching 170,000 CFU/g, followed by the 200 ml (135,000 CFU/g) and 100 ml (114,000 CFU/g) treatments. The control reactor platitude at approximately 13,000 CFU/g. This dose-dependent response confirms that the avocado seed provided essential nutrients (e.g., carbohydrates, minerals) that stimulated the proliferation of indigenous hydrocarbon-degrading bacteria hydrocarbon contaminated soil.

Total Petroleum Hydrocarbon (TPH) Depletion

The TPH degradation profile, presented in (Figure 4), correlates directly with the microbial growth data. The highest TPH removal (75.33%) was achieved in the CT300 bioreactor, where TPH decreased from 3,729.7 mg/kg to 647.7 mg/kg. The CT200 and CT100 reactors achieved 72.17% and 67.76% degradation, respectively. In contrast, the control reactor showed only 38.3% reduction, attributable to natural attenuation. The results unequivocally demonstrate that the efficacy of the bioremediation process is a direct function of the biostimulant concentration.

Relationship between Microbial Growth and TPH Depletion

Figure 5 illustrates the inverse relationship between microbial growth and TPH concentration. As microbial populations increased due to biostimulation, the

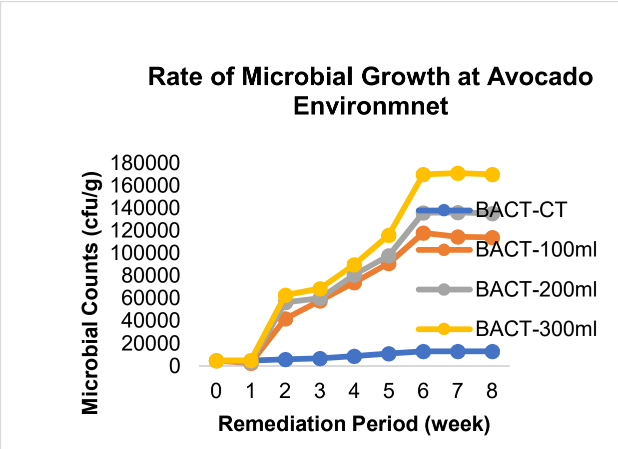


Figure 3: Microbial Growth Rate at the Presence of Fresh Avocado Seed. Microbial Growth Rate at the Presence of Fresh Avocado Seed.

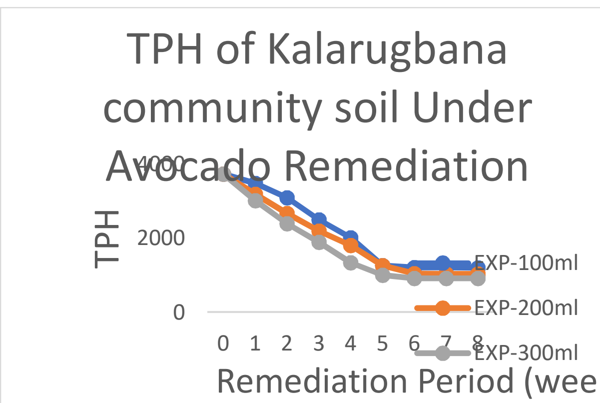


Figure 4: Assessment of TPH Behavior in Avocado Seed Environment. Assessment of TPH Behavior in Avocado Seed Environment.

hydrocarbon substrates were progressively degraded. This synergistic relationship highlights the mechanism through which avocado seed enhances remediation efficiency.

Biokinetic Model Simulation and Validation

The developed biokinetic model was simulated using an optimized equilibrium constant (\(K_{eq} \)) of 0.0083 week⁻¹. The model predictions for substrate performance are shown in (Figure 6), demonstrating the theoretical TPH depletion profiles for different avocado seed applications. A comparison between the model predictions and the experimental TPH data for the 100 ml and 200 ml treatments is presented in (Figures 7 and 8). The close alignment between the simulated curves and the experimental data points validates the model's accuracy. The model successfully captures the first-order kinetic behavior of the degradation process, demonstrating its utility as a predictive tool for assessing bioremediation performance under similar conditions.

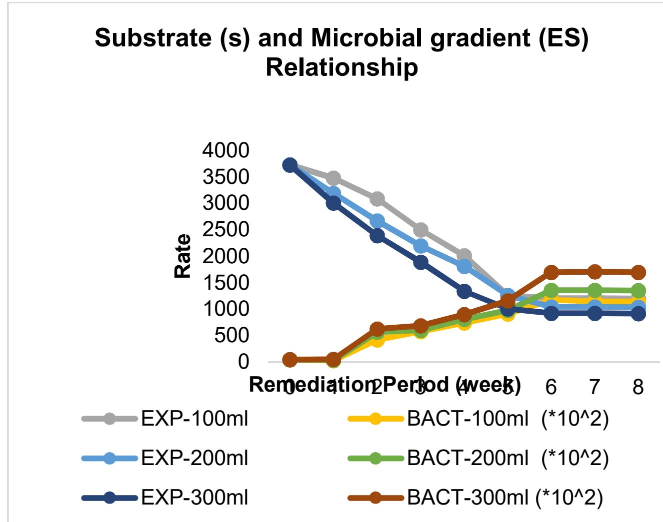


Figure 5: Relationship of TPH and Microbial Growth Rate in Avocado Environment.

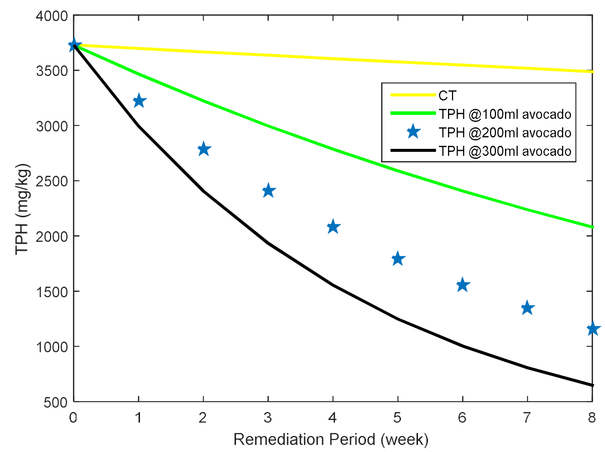


Figure 6: Biokinetic Performance of Kalarugbana Soil Remediation Using Fresh Avocado Seed. Biokinetic Performance of Kalarugbana Soil Remediation Using Fresh Avocado Seed.

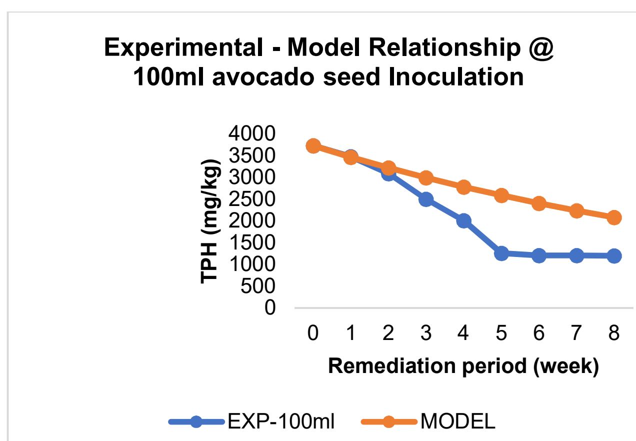


Figure 7: Experimental and Biokinetic Model Correlative Validation. (100ml avocado seed Inoculation).

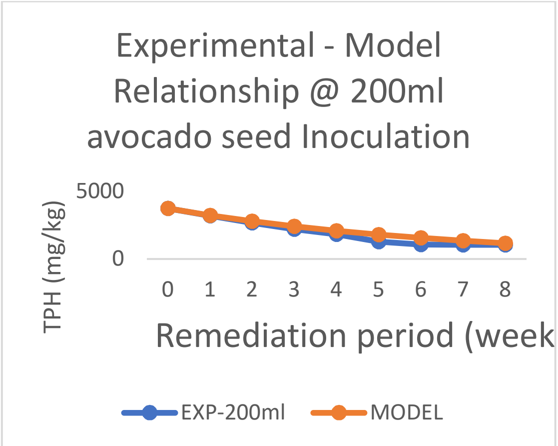


Figure 8: Experimental and Biokinetic Model Correlative Validation (200ml avocado seed Inoculation).

Conclusion and Recommendation

This study successfully demonstrates the high efficacy of fresh avocado seed as a biostimulant for the remediation of crude oil-contaminated soils. The application of avocado seed slurry led to a significant, volume-dependent increase in microbial populations, which directly enhanced the degradation of petroleum hydrocarbons. The key findings are: Avocado seed is a potent, low-cost, and eco-friendly biostimulant. Remediation efficiency is directly scalable to the volume of biostimulant applied. The developed biokinetic model accurately predicts TPH degradation kinetics, providing a valuable tool for process design and optimization.

Based on the findings, the following recommendations are proposed:

1. Field-scale trials should be conducted to validate these laboratory results under natural environmental conditions.
2. The biokinetic model should be tested with other organic waste materials to assess its broader applicability.
3. Future work should focus on isolating and identifying the specific microbial consortia enriched by the avocado seed biostimulation.

Acknowledgments

The author gratefully acknowledge the technical support provided by the Department of Chemical-Petrochemical Engineering, Rivers State University. Special thanks to Prof. Ukpaka C. Peters and Dr. P.N. Ihenyiri for their invaluable supervision and guidance throughout this research. We also acknowledge the Kalarugbana community for providing access to soil and crude oil samples.

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