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Kinetic Studies and Development of Maximum Remediation Time Prediction Models for the Bioremediation of Crude Petroleum Hydrocarbon Polluted Soil

¹Ilaboya, I.R and ²Onaiwu, D.O

¹Department of Civil Engineering, Faculty of Engineering, University of Benin, Benin City, Edo State, Nigeria ²Department of Petroleum Engineering, Faculty of Engineering, University of Benin, Benin City, Edo State, Nigeria

Article Info

Abstract

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https://nipesjournals.org.ng © 2020 NIPES Pub. All rights reserved This paper evaluates the kinetics of bioremediation of petroleum hydrocarbon polluted soil using dead cells microorganism such as yeast, organic substrate such as poultry manure, decayed domestic waste manure and inorganic substrate such as N.P.K fertilizer. Hydrocarbon polluted soil was simulated on a laboratory scale and characterized to determine the particle size distribution, pH, moisture content, total organic carbon, total nitrogen and total hydrocarbon content. For effective treatment, 2:1 mixture (w/w) of the polluted soil and the treatment substrate in addition to 25ml of nutrient agar was utilized. The entire experiment was monitored for a period of twelve (12) weeks and triplicate samples were collected from each setup on a weekly basis and analyzed using Atomic Adsorption Spectrophotometer (AAS) to determine the residual hydrocarbon content. The amount of hydrocarbon removed per time was computed from simple mass balance equation. Results obtained reveal a gradual decrease in total hydrocarbon content (THC) with remediation time. The decrease in THC was also observed to vary with the type of substrate as follows. For yeast; THC reduces from 11.533 to 3.18mg/l. For poultry manure; THC reduces from 11.533 to 3.54mg/l. For NPK; THC reduces from 11.533 to 2.02mg/l. For decomposed waste; THC reduces from 11.533 to 9.17mg/l. The gradual decrease in THC can be traced to the fact that; as the microorganism utilizes the available nitrogen and organic carbon present in the soil and increase in population; they again react with and break down the petroleum hydrocarbon. Based on the model developed, it was observed that; the non-linear regression models perform better than the linear regression method in predicting the rate of hydrocarbon loss with time for the different substrate. More also, the second order kinetic model was observed to have a better fitting of the experimental data.

1. Introduction

A World Bank survey estimated that about 2.3million cubic meters of crude oil is spilt into different media in the Niger Delta Region each year. It has also been claimed that more than 70 per cent of this volume went unrecovered. These unrecovered spills and those that are not properly cleaned up constitute a continuous source of contamination to the subsurface water, soils vegetation and biodiversity. Environmental pollution by petroleum hydrocarbons has become a serious problem in Nigeria and the whole world in general [1]. Invariably, oil spillage damages the soil, water and both plants and animals. Consequent upon its contents of lead, oil pollution renders soils unproductive for years after spillage, reducing the growth performance of plants [2, 3, 4, and 5].

Soil contamination with oil spills is the major global concern today. Soil contaminated with Petroleum has a serious hazard to human health, causes organic pollution of ground water which limits its use, causes economic loss, environmental problems, and decreases the agricultural productivity of the soil [6]. The concern stems primarily from health risks, from direct contact with the contaminated soil; vapour from the contaminants, and from secondary contamination of water supplies within and underlying the soil. The toxicity of petroleum hydrocarbons to microorganisms, plants, animals and humans is well established [7]. The toxic effects of hydrocarbons on terrestrial higher plants and their use as weed killers have been ascribed to the oil dissolving the lipid portion of the cytoplasmic membrane, thus allowing cell contents to escape [8].

Petroleum hydrocarbon (PHC) contamination of soil is a concern for a number of reasons. First, once released into soil, the volatility of PHC can pose a fire or even explosion hazard especially when vapour enters confined spaces. Secondly, contaminants can interfere with the nutrients and water transmission and thus lead to land degradation. Thirdly, weathered petroleum residuals may stay bound to soil particles and be retained in soil for years. Fourthly, although these contaminants may benefit the oil degraders as a carbon source, they are still toxic to the majority of soil biota [9]. PHC pollutants can strongly alter the ecology and the physiology of bacteria and fungi [10]. Lastly, PHC contamination of soil is not only a concern for the soil itself, but is also a potential danger to the plant population [11].

Since the contamination of soil and groundwater by uncontrolled releases of petroleum products has become a significant problem, a number of technologies have been tested to remediate the polluted sites. Large-scale incineration plants have been developed, and incineration of hydrocarbon pollutants is carried out to clean up hydrocarbon contaminated sites. The treatment time is short, but the system requires huge machines and large amounts of heavy oils [12]. Biological treatments for hydrocarbon-degradation have also been investigated and have been found to be less sophisticated natural method of clean-up of hydrocarbon polluted sites, but the low solubility and adsorption of high molecular weight hydrocarbons limits their availability to microorganisms [13, 14]. The specificity of the degradation process is related to the genetic potential of the particular microorganism to introduce molecular oxygen into hydrocarbon and to generate the intermediates that subsequently enter the general energy- yielding metabolic pathway of the cell. The driving force for petroleum biodegradation is the ability of microorganisms to utilize hydrocarbons to satisfy their cell growth and energy needs.

Prediction of the rate of consumption of the available nitrogen (TNC), organic carbon (TOC) and the rate of breakdown of total hydrocarbon content (THC) by microorganism utilizing substrate, will not only help us to understand the performance of the different substrate used, it will also enable us predict the time for maximum remediation that is the time at which the petroleum hydrocarbon that contaminates the soil would have been broken down completely. Accurate prediction of the time to achieve maximum remediation demands a proper modelling of the exact relationship between the amount of petroleum hydrocarbon removed and the treatment time. To get the exact relationship, linear and non-linear modelling has been tested by different researchers. In addition, the purpose of kinetic models is to define the reaction pathway that predefines the entire process of Bioremediation. Kinetic models help us to understand the interrelationship between the rates of hydrocarbon breakdown with time. The order of the reaction (n); which defines the power to which the concentration is raised in the rate limiting step equation is mostly employed to classify kinetic models into zero, first and second order kinetics [11].

2.0 Experimental Procedure

2.1 Samples Collection

The soil sample used for this research was collected from the top surface soil (0-15 cm) in the Faculty of Engineering, behind Petroleum Engineering building, University of Benin, Benin City; using a hand auger. Dead cell microorganism (yeast) was gotten from a pharmaceutical store, organic substrates (poultry manure and decayed waste manure) were gotten from Uniben integrated farm project while the inorganic substrate (NPK manure) was purchased from a chemical store. The crude petroleum hydrocarbon was obtained from Warri Refinery and Petrochemical Company (NNPC).

2.2 Soil sample preparation, simulation and amendment

The sample soil was air dried for approximately one week, homogenized and passed through 2mm pore size sieve and was stored in black polyethylene bag prior to use. 2:1 mixture of the soil and the crude oil was obtained in a plastic bucket. The mixture was properly mixed, covered with a layer of aluminum foil paper and left for four days to stabilize before commencement of treatment. Afterwards, for effective treatment, a 2:1 mixture (w/w) of the polluted soil and the substrate in addition to 25ml of nutrient agar was utilized [15]. The plastic container and its content were open throughout the period of experimentation to allow for the influence of atmospheric oxygen.

2.3 Soil characterization/ physicochemical analysis

Before contamination, the soil sample was characterized after digestion (using 1:1 ratio of 0.25M hydrochloric acid and Nitric acid) to determine the intrinsic properties of the soil. Some of the properties of interest include [16]: total heterotrophic bacterial (THB), pH, electrical conductivity, total hydrocarbon content (THC), total, organic carbon (TOC), total phosphorous (TP), total nitrogen (TN). After contamination, the hydrocarbon polluted soil sample was again subjected to chemical digestion using 1:1 ratio of 0.25M hydrochloric acid and nitric acid. Thereafter, the polluted soil was characterized to determine the same physicochemical properties enumerated as above.

2.4 Experimental setup

Five experimental set-ups were utilized for the study as follows; to the first bucket, a 2:1 mixture of the polluted soil and dead yeast with 25ml of nutrient agar was obtained for further investigation. To the second bucket, a 2:1 mixture of the polluted soil and inorganic fertilizer with 25ml of nutrient agar was obtained for further investigation. To the third bucket, a 2:1 mixture of the polluted soil and poultry manure with 25ml of nutrient agar was obtained for further investigation. To the third bucket, a 2:1 mixture of the polluted soil and organic manure from decayed domestic waste material with 25ml of nutrient agar was obtained for further investigation and the fifth bucket was used as the control set up containing the polluted soil with no treatment substrate and nutrient agar added.

2.4 Efficiency of remediation

The amount of hydrocarbon removed during the series of batch investigation was determined using the mass balance equation of the form

$$q = \frac{v}{m} \left[C_0 - C_e \right] \tag{1}$$

Where: q, defines the hydrocarbon uptake (mg/g); C₀ and C_e: are the initial and equilibrium hydrocarbon concentrations in the digested soil solution [mg/l] respectively; V: is the weight of soil sample taken (g) and M: is the mass of substrate used (g).

The efficiency of hydrocarbon removal (%) was calculated using the mass balance equation of the form.

Removal Efficiency (%) = $\left(\frac{C_0 - C_e}{C_0} \times 100\right)$ (2)

Where: C_0 and C_e are total hydrocarbon content (THC) (mg/l) in digested soil solution before and after treatment respectively.

2.5 Prediction of maximum remediation time

To predict the maximum remediation time, linear and non-linear regression models were developed. For the linear regression model, least square regression approach was utilized while for the nonlinear regression, data fit software was employed.

2.6 Kinetic Studies

To study the kinetics of bioremediation, experimental data obtained were fitted into pseudo-first order kinetic model and pseudo-second order kinetic model. The pseudo first-order rate expression of [17] based on the solid capacity is generally expressed as follows:

$$\frac{dq_t}{dt} = K_1(q_e - q_t) \tag{3}$$

Where:

Qe; and qt; is the amount of petroleum removed at equilibrium and time t, respectively (mg·g-1),

K1; is the rate constant of pseudo first-order adsorption [17].

The linear plots of Log $[q_e - q_t]$ versus time (t) show the appropriateness of the above equation and subsequently the first order nature of the process involved.

The pseudo-second- order equation is also based on the sorption capacity of the solid phase.

$$\frac{dq_t}{dt} = K_2 (q_e - q_t)^2$$
(4)
where :

 K_2 ; is the rate constant of pseudo - second order (gmg⁻¹ min⁻¹)

 $\left(\frac{t}{-}\right)$

The plot of q_t against (t) should give a linear relationship from which q_e and K_2 can be determined from the slope and intercept of the plot [18, 19, 20 and 21].

3.0 Results and Discussion

Result of winsieve classification of the experimental soil is presented in Table 1 while the physicochemical properties is presented in Table 2

Parameters	•	Values
% Clay		7.20
% Silt		72.8
% Fine Sand		9.00
% Medium Sand	11.0	
% Coarse Sand		0.00
% Fine Gravel		0.00
% Coarse Gravel	0.00	
% Cobbles	0.00	

The indication is that, the experimental soil contains very high composition of silt as seen in Table 1. This result justifies the level of carbon and nitrogen found in the soil before the hydrocarbon pollution. Again it also suggested that a reasonable level of microorganism will be present in the soil that will help aid the process of hydrocarbon degradation.

Table 2: Physico-chemical properties of the experimental soil

S/N	Parameter	Mean Test Result of unpolluted soil	Mean Test Result of polluted soil
1	Moisture Content (%)	1.5	25.41
2	рН	6.9	8.03
3	Electrical Conductivity (µs/cm)	59.83	230
4	Total Dissolved Solids (TDS)	33.67	151.8
5	Organic Carbon (g/kg)	16.457	2.16
6	Total Nitrogen (g/kg)	9.023	5.20
7	Total Hydrocarbon Content (mg/kg)	0.00	11.533

Result of Table 2 revealed that, addition of hydrocarbon to soil alters the initial properties of the soil as follows; the soil became highly alkaline with a mean pH of 8.03, high level of conductivity occasioned by the presence of high concentration of dissolved solids as seen from the mean results of conductivity and total dissolved solids $(230\mu$ S/cm and 151.8mg/l) with a drastic reduction in total nitrogen and total carbon concentration caused by a slight increase in the soil petroleum hydrocarbon content due to pollution.

When the effect of bioremediation on hydrocarbon polluted soil was studied in terms of the total hydrocarbon content (THC), result obtained is presented in Figure 1.

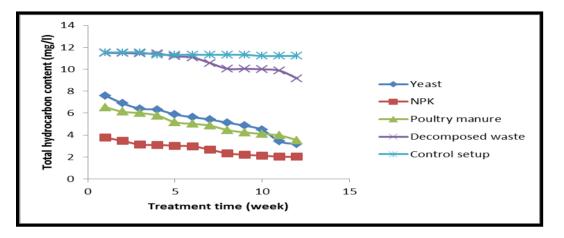


Figure 1: Variation of total hydrocarbon content with treatment time

It was observed during the entire period of experimentation that the total hydrocarbon content (THC) decreases gradually for all the substrate used due to degradation by the microorganism present in the soil. As the microorganism utilizes the available nitrogen and organic carbon nutrient present in the soil and increases their population, they again react and break down the agent that causes the pollution which is the petroleum hydrocarbon. The breakdown of petroleum hydrocarbon occasioned by increase in the population of the soil microorganism consequently results to a gradual decrease in petroleum hydrocarbon concentration as shown in Figure 1. Result of the control setup shows that, there was no remediation since the microorganism do not have supplementary nutrient to resist the effect of hydrocarbon pollution. The breakdown of petroleum hydrocarbon by the available microorganism results in the formation of carbonzylic acid as by product.

To study the kinetics of bioremediation, experimental data obtained were fitted into the first and second order kinetic model. The kinetic model was employed to study the time dependent effects of the process of remediation. The main focus is to monitor the change in the concentration of hydrocarbon with treatment time in other to understand the nature of chemical reaction involved in the biodegradation of petroleum hydrocarbon. Results of the kinetic models are presented in Figure 2 and 3 respectively.

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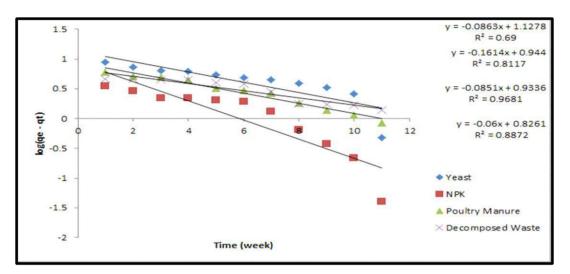


Figure 2: Pseudo-first order kinetic for the bioremediation of hydrocarbon polluted soil

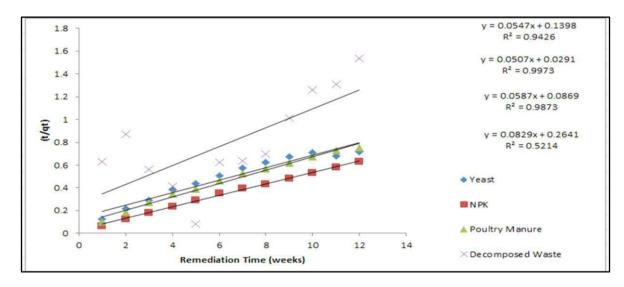


Figure 3: Pseudo-second order kinetic for the bioremediation of hydrocarbon polluted soil

To compute the kinetic parameters and determine the kinetic model that best explains the experimental data, non-linear regression software (NLREG) was employed and results obtained is presented Table 3.

Table 3: Computed Parameters for pseudo-first and pseudo-second order kinetic mode	Table 3: Com	puted Parameter	s for pseudo-first an	d pseudo-second orde	er kinetic model
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PseudoFirst Order Kinetic Parameters	Yeast	NPK	Poultry manure	Decomposed waste
R ²	0.690	0.8117	0.9681	0.8872
q _e	3.08894	2.5703	2.5435	2.2844
K ₁	0.1987	0.3717	0.1959	0.13818

PseudoSecond Orde Kinetic Parameters	r Yeast	NPK	Poultry manure	Decomposed waste
R ²	0.9426	0.9973	0.9873	0.671
q _e	12.1977	18.7426	13.0893	39060.64
K ₂	-204997.3	0.1967	-210873.8	2.0040E-010

On the kinetics that best explain the experimental data, the values of the linear coefficient of determination was employed as bases for judgment and result of Table 3 shows that the second order kinetic model best fit the experimental data when yeast, NPK and poultry manure were used as the treatment substrate. For decomposed waste as treatment substrate, experimental data obtained was best explained by the first order kinetic model.

Application of linear regression approach to predict the time for maximum remediation of petroleum hydrocarbon and time for maximum utilization of TNC and TOC yielded the results presented in Figures 4, 5 and 6 respectively.

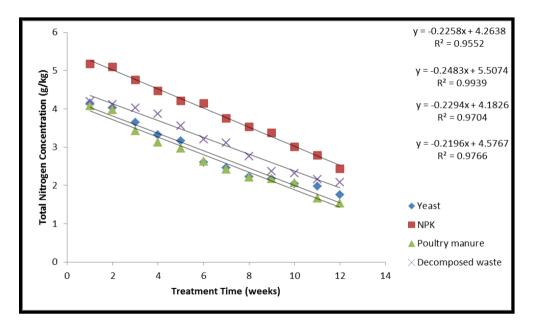


Figure 4: Prediction of total nitrogen content utilization by linear regression model

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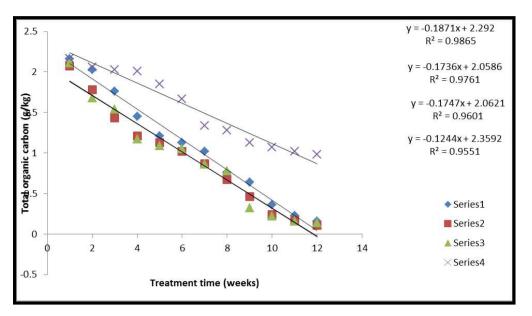


Figure 5: Prediction of total organic carbon utilization by linear regression model

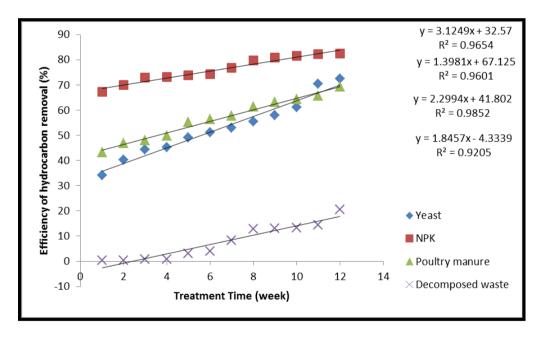


Figure 6: Prediction of hydrocarbon removal by linear regression model

The adequacy of the linear regression model was validated using the linear coefficient of determination presented in Table 4.

Table 4: Parameters of Linear Regression Model

S/No	Parameter	Substrate	Prediction Equation	R ²
		Yeast	Y = -0.2253x + 4.2638	0.9552
		NPK	Y = -0.2483x + 5.5074	0.9939

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1	TNC	Poultry manure	Y = -0.2294x + 4.1826	0.9704
		Decomposed waste	Y = 0.2196x + 4.5767	0.9766
		Yeast	Y = -0.1871x + 2.292	0.9865
		NPK	Y = -0.1736x + 2.0586	0.9761
2	TOC	Poultry manure	Y = -0.1747x + 2.0621	0.9601
		Decomposed waste	Y = -0.1244x + 2.3592	0.9551
	I			I
		Yeast	Y = 3.1249x + 32.57	0.9650
3		NPK	Y = 1.3981x + 67.125	0.9601
	THC	Poultry manure	Y = 2.994x + 41.802	0.9852
		Decomposed waste	Y = 1.8457x + 4.339	0.9205

For the non-linear regression approach, results obtained is presented in Tables 5, 6, 7 and 8 respectively.

Table 5: Non-linear prediction of total hydrocarbon content using yeast

Results from pr	Results from project "Untitled5"					
Equation ID: a*	*x^3+b*x^2+c*x+d					
Model Definition	n:					
$Y = a^{x^3+b^{x^4}}$	^2+c*x+d					
Number of obse	ervations = 12					
	sing observations = 0					
Solver type: No						
Nonlinear iterat						
	near iteration limit =10					
	linear iterations perform	ed = 2				
	nce = 0.0000000001					
	als = 3.1086244689504					
	ual = 2.5905203907920					
	of Squares (Absolute) =					
	of Squares (Relative) =					
	of the Estimate = 0.16					
Coefficient of M	Aultiple Determination (R^2) = 0.9891629855				
Proportion of V	ariance Explained = 98	.91629855%				
		nination (Ra^2) = 0.98509	91051			
Durbin-Watson	statistic = 2.88307381	961129				
	Regression Variable Results					
	alue		t-ratio	Prob(t)		
		1.49994655534858E-03		0.00597		
		2.95808540845157E-02		0.0097		
		0.168888444304241	-4.947188654	0.00112		
d 8.2	27535353535354	0.264114139974194	31.33248957	0.0		

Table 6: Non-linear prediction of total hydrocarbon content using NPK

	n project "Untitled6"			
	: a+b*x+c*exp(x)			
Model Defini				
Y = a+b*x+c	c*exp(x)			
Number of o	bservations = 12			
Number of n	nissing observations = 0			
Solver type:				
Nonlinear ite	eration limit = 250			
Diverging no	nlinear iteration limit =10			
	onlinear iterations perform			
	erance = 0.0000000001			
Sum of Resi	iduals = 1.332267629550	19E-15		
Average Res	sidual = 1.110223024625	16E-16		
Residual Su	im of Squares (Absolute)	= 0.108450832017433		
	im of Squares (Relative) =			
	ror of the Estimate = 0.10			
Coefficient o	of Multiple Determination ((R^2) = 0.9719770119		
	f Variance Explained = 9			
Adjusted co	efficient of multiple deterr	mination (Ra^2) = 0.96574	496813	
	son statistic = 1.3384900			
Regression	Variable Results			
Variable	Value	Standard Error	t-ratio	Prob(t)
a	3.85689344940645	7.54618897441693E-02	51.11048057	0.0
b	-0.177336279764772	1.23391143322678E-02	-14.37188075	0.0
с	1.82352698382186E-06	9.31154701799816E-07	1.958350186	0.08186

Table 7: Non-linear prediction of total hydrocarbon content using poultry manure

E and in ID	- *··· • · · · · · · · · · · · · · · · ·	0		
	a*x^5+b*x^4+c*x^3+d*x^	Z+e"x+t		
Model Defin				
$Y = a^x+5+b$	o*x^4+c*x^3+d*x^2+e*x+f			
	bservations = 12			
	nissing observations = 0			
Solver type:				
Nonlinear ite	eration limit = 250			
Diverging no	nlinear iteration limit =10			
	onlinear iterations perform	ned = 11		
Residual tol	erance = 0.0000000001			
Sum of Res	iduals = 1.0554801477269	94E-10		
Average Res	sidual = 8.7956678977244	9E-12		
Residual Su	im of Squares (Absolute)	= 8.24328157136975E-02	2	
Residual Su	im of Squares (Relative) =	8.24328157136975E-02		
Standard Er	ror of the Estimate = 0.11	7212638477894		
Coefficient of	of Multiple Determination (R^2) = 0.9919237092		
Proportion of	f Variance Explained = 99	0.19237092%		
	efficient of multiple detern		34669	
Durbin-Wats	son statistic = 2.53493818	3069233		
Regression	Variable Results			
Variable	Value	Standard Error	t-ratio	Prob(t)
а	-1.45267722448574E-04	1.39380916565336E-04	-1.042235379	0.33747
b	4.31363979092499E-03	4.54596073314522E-03	0.9488950838	0.37931
с	-4.52300579268935E-02	5.45079403057681E-02	-0.8297884248	0.43842
d	0.204977289854517	0.29289706747637	0.6998270472	0.51024
е	-0.679494583855166	0.67884349487215	-1.000959115	0.35549
f	7.04545454545177	0.512138879425224	13.75692186	0.00001

Table 8: Non-linear prediction of total hydrocarbon content using decomposed waste

Results from project "Untitled8"								
	a+b*x^2*ln(x)+c*x^2.5							
Model Defini								
$Y = a+b*x^2$?*ln(x)+c*x^2.5							
Number of o	bservations = 12							
Number of n	nissing observations = 0							
Solver type:	Nonlinear							
Nonlinear ite	eration limit = 250							
Diverging nonlinear iteration limit =10								
Number of nonlinear iterations performed = 11								
Residual tolerance = 0.000000001								
Sum of Residuals = -3.5527136788005E-15								
Average Residual = -2.96059473233375E-16								
Residual Sum of Squares (Absolute) = 0.318312872802522								
Residual Sum of Squares (Relative) = 0.318312872802522								
Standard Error of the Estimate = 0.188064076787828								
Coefficient o	Coefficient of Multiple Determination (R^2) = 0.954765131							
Proportion o	Proportion of Variance Explained = 95.4765131%							
		mination (Ra^2) = 0.9447	129379					
Durbin-Wats	son statistic = 1.7504037	5965425						
Regression	Variable Results							
	Value	Standard Error	t-ratio	Prob(t)				
a	11.4059422690064	7.77258632220407E-02	146.745778	0.0				
b	-0.091113962128276	3.49754705701922E-02	-2.605081809	0.0285				
С	6.13124585008945E-02	2.52989729147631E-02	2.423515718	0.03839				

The estimated coefficient of determination of the non-linear model developed is presented in Table 9.

Table 9: Parameters of Non-Linear Regression Model

S/No	Parameter	Substrate	Model Equation	R ²
1		Yeast	$Y = a + bx^2 + c\ln(x^2)$	0.9907
	TNC	NPK	$Y = a + bx^{2.5} + c\ln(x^2)$	0.9960
	INC	Poultry manure	$Y = [a + bx^{0.5} + c \ln(x)] / x^2$	0.9910
		Decomposed waste	$Y = a + bx^{2.5} + cx^3$	0.9942
2		Yeast	$Y = ax^{5} + bx^{4} + cx^{3} + dx^{2} + ex + f$	0.9876
	TOC	NPK	$Y = ax^4 + bx^3 + cx^2 + dx + e$	0.9871
	IOC	Poultry manure	$Y = [a + bx^{1.5} + c]/2$	0.9811
		Decomposed waste		0.9933

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		Yeast	$Y = ax^3 + bx^2 + cx + d$	0.9892
3	ТНС	NPK	$Y = a + bx + c \exp(x)$	0.9720
		Poultry manure	$Y = ax^{5} + bx^{4} + cx^{3} + dx^{2} + ex + f$	0.9919
		Decomposed waste	$Y = a + bx^2 \ln(x) + cx^{2.5}$	0.9447

Results of the linear and non-linear regression analysis presented in Tables 4 and 9 reveals that the non-linear regression modeling gave a better interpretation of the experimental data and hence can be employed to predict the maximum remediation time coupled with the rate of nutrient depreciation. For example, in the study of total hydrocarbon breakdown using NPK, a non-linear regression model equation of the form $[Y = a + bx + c \exp(x)]$ where x is time and y is amount of hydrocarbon degradation] was generated and the goodness of fit statistics indicates that (r² = 0.9720) as against the linear regression model with (r² = 9601). Thus, the non-linear regression model equation was adopted as the controlling equation which can be employed to predict the time to achieve total breakdown of the petroleum hydrocarbon present.

4.0 Conclusion

In this study, linear and nonlinear regression modelling on the application of yeast, inorganic substrate such as NPK, organic substrate such as poultry manure and decomposed waste in the bioremediation of hydrocarbon polluted soil have been done. The models developed can be employed to predict the maximum remediation time for hydrocarbon polluted soil including the maximum utilization time for total nitrogen content and total organic carbon content. In addition, kinetic studies on the application of yeast, inorganic substrate such as NPK, organic substrate such as poultry manure and decomposed waste in the bioremediation of hydrocarbon polluted soil have been successfully investigated. The outcome of the study shows that; the non-linear regression models perform better than the linear regression method in predicting the rate of hydrocarbon loss with time for the different substrate and the second order kinetic model was observed to have a better fitting of the experimental data.

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