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MODELLING BIOREMEDIATION OF CRUDE OIL CONTAMINATED SOIL

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Abstract

The work is aimed at remediating a crude oil polluted site using different treatment materials and option A to arrive at the best treatment option. The treatment were executed using microorganisms and locally formulated NPK fertilizer. The mean TPH Field data generated from the delineated site was collected for a period of 84 days at (0 - 14 days) interval and the mean values were subjected to bioremediation kinetics. Mathematical models were derived and predicted results from the models were compared to field data.

Keywords*: Bioremediation, Total Petroleum Hydrocarbon, Delineated;* Microbial growth*, NPK fertizer*

1.0 INTRODUCTION:

Bioremediation has been identified as one important methods of decontaminating crude oil polluted soil (Adams, 2015, Akpan, 2014.and Mohammed, 2017). Total petroleum hydrocarbon (TPH) was seen by previous researchers as one of the major pollutants that deplete the nutrient parameters in most of the soil environments polluted with Crude oil (Nwakwasi, 2016, Onwuka, et al. 2012). This attracted more researchers to see how the problem can be solved especially at this period when there is urgent need to recover polluted environments for economic reusability and health related problems recorded by the inhabitants of the affected areas Prasad, (2016, Varjani, 2017, Yavari, et al. 2015). Petroleum hydrocarbon Pollution and its remediation process has cost the Oil producing nations a lot of resources in an attempt to remediate any affected area (Siles, 2018). This has led to several searches for more affordable methods of reclamation processes including modelling so as to provide the operators and the Engineers with the requisite tool in designing a typical bioremediation site (Ukpaka, 2013, and Uwadiae, and Obasi 2021). To provide these affordable methods of solving these environmental related issues, many researchers have been involved in environmental studies using Models (Smith, E., et al 2015, Ukpaka, 2013, and Somtrakoon, et al (2014). The Field operation to determine the concentration usually takes time and resources to formulate the model (Thapa,. & Kumar, 2012, Uwadiae, and Obasi, 2021).

The research is aimed at developing equations describing the rates of microbial growth and crude oil (TPH) consumption by the microbes in the treated crude oil contaminated soil.

2.0 MATERIALS AND METHODS

The field work was conducted for approximately twelve weeks and at the period when weather condition is considered mild. The materials used in the study are of analytical grade. Locally made organic manures (NPK) and indigenous

microbes were used. Physicochemical characteristics of the soil in the study area were conducted. Different methods of bioremediation were applied for a period of 84 days. After random spot collection at different depths, the samples were homogenized and packaged in a well labelled polythene bag and immediately taken to laboratory for analysis at every time interval. The mean values are computed and recorded for the final analysis.

2.1 METHODS OF FIELD WORK AND PROCEDURE

Plot A: Combination of microorganisms (*Pseudomonas genera*), NPK fertilizer and tilling.

Plot A0: No treatment. (Control).

2.2 ADDITION OF INDIGENOUS MICROORGANISMS, FERTILIZER AND TILLING

Indigenous microorganisms (*Pseudomonas genera*), (NPK) fertilizer was applied to Plot A and tilled.

2.3 SOIL SAMPLING AND CHARACTERIZATION

Soil samples was obtained from the topsoil and from the top soil. de 0.5m and 1.0m depth at random spots. The samples were homogenized, put in a well labeled polyethylene bag and taken immediately to the laboratory for analysis. This was done in three replicates, and the mean result of the three replicates was used for data analysis. Soil sampling and analysis was conducted every 2 weeks for 12 weeks as shown in Table 1.

2.3.1: ORGANIC CARBON CONTENT: The organic carbon was measured using Walkley and Black Method.

2.3.2: NITROGEN AND PHOSPHORUS NUTRIENTS CONTENTS: The nitrogen content was measured using Kjeldahl steam distillation method, while the phosphorus content was measured using total phosphorus method.

2.3.3: SOIL PH: The soil pH was measured using a 1:1 water ratio with the aid of a glass Electrode pH meter.

2.3.4: SOIL DENSITY: The density of particles was measured using 60mL Pycnometers

2.4 ENUMERATION AND SCALING UP OF MICROORGANISMS

The population of microorganisms present in the soil was estimated by placing the soil samples in contact with sterile normal saline (0.85% NaCl) solution. This mixture was placed in a shaker for about 30 ± 5 minutes to extract the microorganisms present in the soil. The total heterotrophic bacteria count was done using the surface spread method since the efficiency of any bioremediation process depends to a large extent on the amount of microorganisms present. The population of microorganisms at the spilled site was very low prior to bioremediation therefore, inoculum was added to boost the process. The inoculum was developed from hydrogen utilizing genera from the site, with the addition of crude oil as the source of carbon and appropriate nutrients.

First order kinetic equation ($\frac{dc}{dt} = -KC$) was redefined to $\frac{1}{C} \times \frac{dc}{dt} = -K$ to obtain k values of Total Petroleum Hydrocarbon.

2.5 MATHEMATICAL MODELLING OF CRUDE OIL REMOVAL FROM CONTAMINATED SOIL

Base on the application of Law of conservation of Mass and Bio-kinetics, batch reactor system can be used to develop model of bio-consumption of crude oil from the soil. The biochemical reaction resulting from micro-organism consumption of crude oil in a contaminated soil is given as:

Crude oil + O_2 + Nutrients = CO_2 + H_2O + New Cells + Energy (1)

The law of conservation of mass for this biochemical reaction is given as:

$$R_a = R_{fin} - R_{fout} - R_c \quad (2)$$

Where, R_a = Rate of crude oil accumulation in the soil, R_{fin} = Rate of crude oil flow into the soil, $fout$ = Rate of crude oil flow out the soil, R_c = Rate of microbial consumption of crude oil

Considering microbial consumption of the crude oil in the soil as a batch process, the rate of crude oil flow into the system, R_{fin} = 0, and the rate of crude oil flow out of the system, R_{fout}= 0. Therefore Equation 2 reduces to:

$$R_a = R_c \quad (3)$$

The microbial concentration increases with time, as the total rate of change in microbial concentration is described as:

$$\frac{dX}{dt} = \mu x_t \quad (4)$$

Where X_t = Microbial concentration (mg/L) at time t, and μ = Specific growth rate constant (h^{-1}).

Also, the rate of crude oil (TPH) consumption is described as:

$$-\frac{dS}{dt} = \frac{\mu X_t}{Y} \quad (5)$$

Where S = Oil (TPH) concentration (mg/L), and Y = Microbial growth yield (h^{-1}).

Using Monod Equation to relate the specific growth rate of the microorganism to crude oil (TPH) concentration in the contaminated soil is given as:

$$\mu = \frac{\mu_m S}{K_s + S} \quad (6)$$

Substituting Equation 6 into Equations 4 and 5 gives:

$$\frac{dX}{dt} = \left(\frac{S}{K_s + S}\right)\mu_m X_t \quad (7)$$

$$\frac{dS}{dt} = -\frac{\mu_m}{Y}\left(\frac{S}{K_s + S}\right)X_t \quad (8)$$

The microbial yield is defined as:

$$Y = \frac{dX}{dS} = \frac{X_t - X_0}{S_0 - S_t} \quad (9)$$

Therefore,

$$X_t = Y(S_0 - S_t) + X_0 \quad (10)$$

Substituting Equation 10 into Equation 8 gives:

$$\frac{dS}{dt} = \left[-\frac{\mu_m}{Y} \left(\frac{S}{K_s + S} \right) \right] Y(S_0 - S_t) + X_0 \quad (11)$$

Multiplying Equation 11 through gives:

$$\frac{dS}{dt} = \left[\frac{\mu_m Y S_t^2 - \mu_m Y S_0 S_t - \mu_m X_0 S_t}{Y K_s + Y S} \right] \quad (12)$$

Equations 7 and 12 are sets of first-order differential equations describing the rates of microbial growth and crude oil (TPH) consumption by the microbes in the treated crude oil contaminated soil.

2.6 DETERMINATION OF SPECIFIC GROWTH RATE (μ), MAXIMUM SPECIFIC OIL CONSUMPTION RATE (μ_m), HALF-SATURATION COEFFICIENT (K_s) AND YIELD (Y) FROM FIELD DATA.

The microbial specific growth rate (μ) resulting from consuming of the contaminant (crude oil) in the soil is obtained by re-expressing Equation 4 as:

$$\mu = ln\left(\frac{X_t}{X_0}\right)\frac{1}{t} \quad (13)$$

By inversion of Equation 6 gives:

$$\frac{1}{\mu} = \frac{K_s}{\mu_m}\frac{1}{S} + \frac{1}{\mu_m} \quad (14)$$

Plotting Equation 13 will give a straight-line graph where $\frac{K_s}{\mu_m}$ is the slope, and $\frac{1}{\mu_m}$ is the intercept of the graph, respectively. The growth yield Y is gotten from Equation 9.

2.7 NUMERICAL SOLUTION FOR MODEL EQUATIONS

Equations 7 and 12 will be cumbersome to solve using analytical techniques due to the interaction effect between the variables and the need to solve them simultaneously. Therefore, the fourth order Runge-Kutta (R-K) method was used to solve them, as it offers straightforward computational procedures and accurate solutions. The model equations were solved using the R-K method, which is an explicit algorithm that solves initial value problems by evaluating the function of $f(x)$ at points between x_i and x_{i+1}. The model was discretized by the algorithm for the R-K method and solved using MATLAB program.

3.0 RESULTS AND DISCUSSION

3.1 TPH AND MICROBIAL CONCENTRATIONS FROM THE TREATMENT OPTIONS

The results of different treatment techniques applied to the delineated sites within 0 to twelve weeks duration are shown in Tables 1 and 2. The results were used to determine other required parameter values using bio-kinetics equations.

Table 1 Mean Total Petroleum Hydrocarbon (TPH) Concentration

Time t (days)	0	14	28	42	56	70	84
Mean TPH Conc (mg/kg)	39848.5	31865.7	4718.12	3485.48	2015.07	1139.7	941.5

Table 2: Total Heterotrophic Bacteria Count

Option	Sampling Period (weeks)						
	0	2	4	6	8	10	12
	($x10^5$Cfu/g)						
A0	80	200	1320	2500	3300	3950	5860
A	750	3500	14670	223000	368000	456000	102000

The biokinetic constants; maximum substrate utilization rate (k_m) and half saturation constant (k_S), are obtained by plotting $1/\mu$ against $1/S_t$ using data showed in Table 3.

Table 3. Determination of Biokinetic Constants from Field data of Treatment Option A.

T	X_t	S_t (mg/kg)	μ	$1/S_t$	$1/\mu$	$X_t - X_0$	$S_0 - S_t$	Y
0	8530	39848.5		2.5095E-05	0	0	0	0
14	34750	31865.7	0.10032	3.1382E-05	9.96732	26220	7982.8	3.28456
28	273300	4718.12	0.07365	0.00021195	13.57648	238550	27147.58	8.78716
42	3600000	3485.48	0.06138	0.0002869	16.29095	3326700	1232.64	2698.84
56	4.7E+07	2015.07	0.04603	0.00049626	21.72151	43819000	1470.41	29800.5
70	6.3E+08	1139.7	0.036907	0.00087742	27.09485	5.81E+08	875.37	663241
84	9.6E+09	941.5	0.032464	0.00106213	30.80333	8.97E+09	198.2	4.5E+07

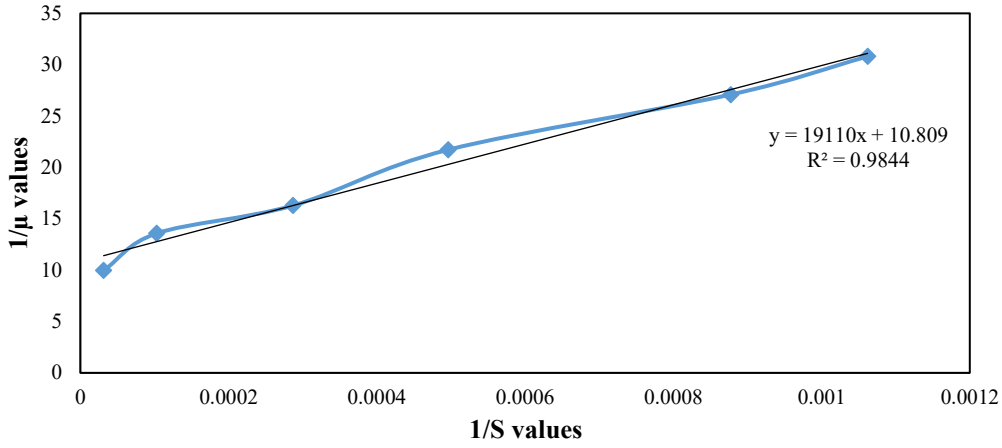


Figure 1: Plot of 1/μ vs 1/S

Figure 1 is a plot of 1/μ against $1/S_t$, where the slope of the equation, m corresponds to (k_s / k_m) and the intercept, c corresponds to $(1/k_m)$, which are obtained from the equation of the line, $y = 1911x + 10.809$, having the best fit given as $R^2 = 0.9944$, which implies that the results are 98.442% reliable. From the equation of the line, the maximum substrate utilization rate (k_m) is given as 0.11 day^{-1} and the half saturation constant, (k_s) is 1911.0 mg/l. which indicates high microbial affinity for crude oil utilization.

Table 4 shows the Model predicted results and the Field data for TPH Removal. The comparison shows a deviation of 1.13% of the model predicted result from the field data, which implies that the developed model can be used to predict the fate of the bioremediation process.

Table 4: Model Results and Field Data for TPH Removal.

T (days)	TPH (Model Results) Mg/kg	TPH (Field Results) Mg/kg	%Deviation
0	39848.5	39848.5	
14	31794.96	31865.7	
28	9473.34	4718.12	
42	5785.48	3485.48	
56	2271.23	2015.07	
70	1439.7	1139.7	
84	952.2	941.5	1.130063

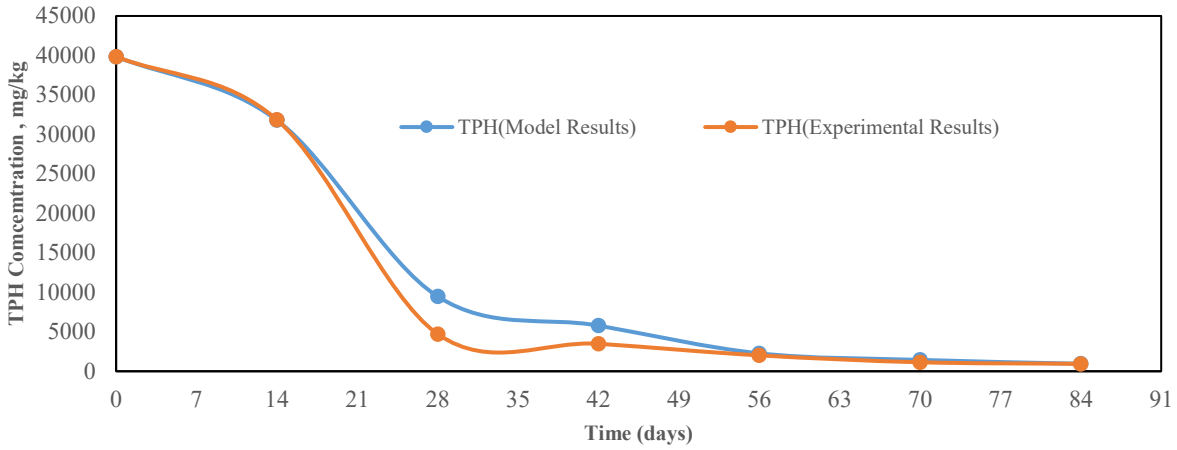


Figure 2: Plot of TPH (Model and Experimental) results against Time

Figure 2 is a plot of the model predicted results and the field data for the best treatment (Option A). Figure 2 shows that the model can predict the bioremediation process reasonably.

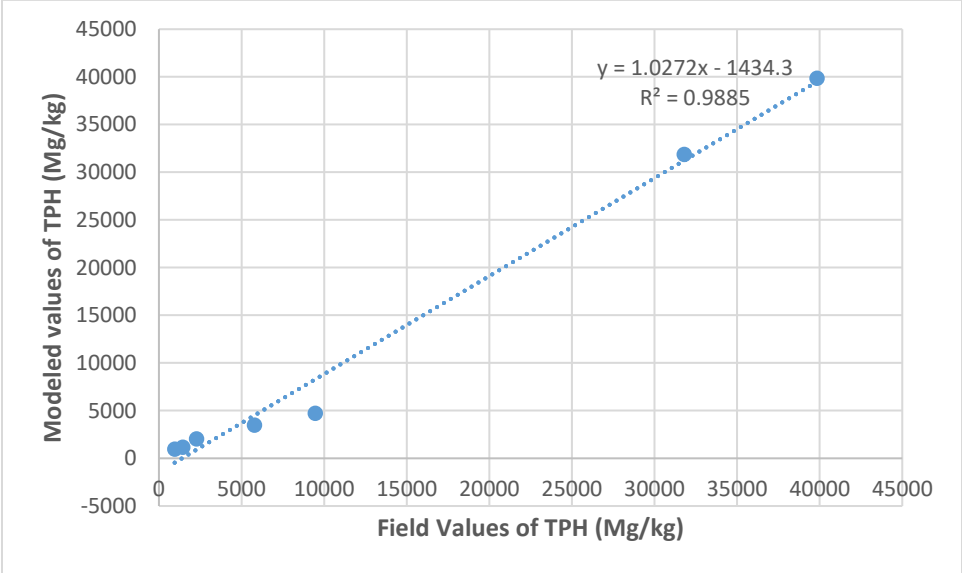


Figure 3: Plot of Modeled values of TPH against Experiment values of TPH

Figure 3 is a plot of the modeled results against the field data for the best treatment (Option A). Figure 3 demonstrated strong correlation between the modeled values and field data values of TPH determined for the remediated soil. The established correlation reveals the effectiveness of the developed model in predicting the bioremediation process reasonably.

4.0 CONCLUSION

From the Total Petroleum Hydrocarbon model process, it can be concluded that decrease in TPH is indicative of the ability of indigenous microorganisms to adapt to the presence of the contaminants and bring about their transformation to reduce levels of contamination in the soil. Reduction in Carbon content is attributed to microorganism degradation of major constituent of carbon and hydrogen to form CO_2 and H_2O. Escape of CO_2 caused reduction in Carbon content of the soil. It was noticed that when the toxicants were degraded by the microbes, percentage nitrogen started improving. The increase in microbial population to soil contaminated with crude oil increases the rate of utilization of percentage Nitrogen. However, variation of microbial growth with time showed considerable improvement. At the first three weeks of bioremediation, microorganism population was very low but after addition of inoculum, there was acceleration in growth of microbes with respect to time. Maximum growth of microbes occurred at the eight weeks in treatment option A before reduction was noticed again in the tenth week. The early growth is attributed to the activities of the injected microorganisms while reduction in the tenth week is attributed to die off rate which occurs when microbes degrade contaminants.

Following the field data, it can be concluded that Combination of NPK Fertilizer, and viable indigenous microbes and tilling is effective treatment of crude oil impacted soil and the developed mathematical model can be applied in predicting the fate of the bioremediation process.

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