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Remediation of crude oil in loamy soil: the integration of improved oil palm fiber (*Tekena Species*) dried in dark environment

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ABSTRACT

Investigation was conducted to demonstrate the integration of improved oil palm fiber (*Tekena* Species) dried in dark environment for the remediation of loamy soil polluted with crude oil. Analysis was conducted to determine the characteristics of the effectiveness of the improved oil palm fibre (*Tekena* Species) dried in dark environment on the degradation of the crude oil in loamy soil environment using X-ray fluorescence spectrometer (GC).The following elements were identified from the *Tekena Species* Mg, P, Si, S, K, and Ca were obtained within the energy level of > 0 to < 250J, Ti, Mn, Fe, Co, Ni, Cu and Zn with energy level range of > 250J to < 590J, W, Au, Pb, Rb, Zr, Nb, and Mo with energy level range of > 600J to< 1200J and Ag, Cd, Sn and Sb with energy level range of > 1400J to < 1800J. The micro-organism isolated and identified were six different fungi species with a population of $1.2x10^5 CFU.g^{-1}$ for *Tekena Species*. The bacteria isolated and identified five different species with a population of $9.0x10^6 CFU.g^{-1}$ for

Tekena Species. It is observed that species are very effective when used for bioremediation of polluted soil environment. The Total Petroleum Hydrocarbon (TPH) in the loamy soil sample was examined for 0 to 84 days to ascertain the degree of degradation upon the influence of improved oil palm fibre (*Tekena* Species) dried in dark environment and the characteristics to improve the level of restoration of the polluted loamy soil was encouraging. A model was developed to determine the rate of degradation of pollutants with time. The result from the model validate the experiment with improved oil palm fibre (*Tekena* Species) dried in dark environment and the degree of degradation is rated in percentage as 76.45%.

Keywords

Remediation, crude oil, loamy soil, integration improved, oil palm fiber (*Tekena species*), dried dark environment

1. INTRODUCTION

Bioremediation is a biotechnological approach of rehabilitating areas degraded by pollutants or otherwise damaged through mismanagement of ecosystem (Binet, *et al;* 2000, Obahiagbon, 2012). Biotechnology applied a set of scientific skills that utilize living organisms to produce, improve and degrade plants and animals product. Various works have been done by researchers on bioremediation of crude oil polluted site that covers on biotransformation, biodegradation and mineralization. Details are reviewed in this work.

Biotechnology, otherwise called an engineering application of microbial ecology and process design (Chukwuma, *et al;* 2012., Ogbo et al., 2009). This involves the design of special machines or equipment (reactor or bioreactors) for the development of specific organism for specific application and purpose. Hence it involves several disciplines such as mathematics, biology, chemistry, physics, Agriculture, Education, health care and engineering. This leads to mathematical modeling which cut across these disciplines with a logical framework to resolve environmental problem affecting the ecosystem. These models are tools that are utilized for explaining the cost, effectiveness and reliability of any clean-up method and control (Onwurah and Alumanah,2005.,Ukpaka and Ogoni, 2017).

Bioremediation as a natural process that transforms contaminants into non-hazardous chemical, which is similar to bacterial remediation which use bacteria to breakdown and finally consume contaminants (Chamberlain, 2012). The microorganisms transform these harmful substances into non-toxic carbon(iv) oxide, water and fatty acids. Naturally occurring microorganisms, enzymes, chemicals and weather on their own eliminate both natural and man-made pollution. Microorganisms, like all living creatures require nutrients such as nitrogen, phosphate, carbon, water and environment to grow and survive. Nutrients enhance the activity of indigenous organism provided the geographical and climatic conditions are observed. When these conditions are present, some micro-organisms will break down organic contaminants as a source of carbon for energy and growth. Around each hydrocarbon molecule is a life support environment for microbes to flourish which consists of water and nutrient. Surfactants, emulsifiers and enzymes present break the contamination down so that the microbe can consume the carbon. This result in the contamination being metabolized by the microbes into harmless carbon (iv) oxide, water and fatty acids. When the food source is depleted and microbes die off then the contaminated site is restored (Kyang et al., 2004., Jones and Edington, 2005).

Crude oil with contaminant containing carbon chain molecules like fuel oil, toluene and in cases chlorine are too complex and toxic for most microbes to metabolize (Jason, 2000). Naturally occurring micro-organisms can be cultured to attack these components. Microbes along with enhancer like oxygen are put in contact with the contaminants; they attack the complex hydrocarbon molecules and break them down into harmless by products.

Biotransformation is a process where the contaminant molecules are altered into less or non-hazardous molecules. Biodegradation involve the breakdown of organic substances in small organic or inorganic molecules. Mineralization is a process where there is a complete biodegradation of organic material into inorganic constituents such as carbon (iv) oxide or water (Ukpaka and Ogoni, 2017).

The choice of the strategy depends on the soil type and correct application. Normal soils are replete with a huge range of naturally occurring microbes, which interact in a complex micro-ecosystem. Concentrated hydrocarbons and solvents may kill almost all microbes; some species will feed on and degrade many less concentrated contaminants. There is man-made attempt at improving the speed and efficiency of natural bioremediation.

Corrective action plans using bioremediation need to be evaluated on a site-by-site basis because microbial action is controlled by site conditions and which may be influence based on environmental factors (Mitchell, 2012). The balance between the damage to the environment caused by the hydrocarbon and the potential damage that may be caused by bio-remediating microbes and even the cost must be determined.

2. MATERIALS AND METHODS

This session details the material used in the experiment, the method, adopted, the model developed that predict the rate of degradation of contaminant with time.

Equipment, Materials and Reagents Used

The following equipment, materials and reagent were used during this research work as stated; Oil palm fibres (*Tekena species*) dried in dark environment, crude oil (Bonny light), glassware, weighing beam, stop clock, bunsen burner, loamy soil, P^H meter, skyray instrument (X-ray fluorescence spectrometer), flame ionization detector, bright field microscope, polythene bag/foil paper, Incubator, chromatographic column, autoclave, fridge, pentane, ethanol, iodine, hydrogen perioxide.

Experimental Procedures

Sample Collection

The sample used in this research work was collected as stated. The improved oil palm fiber (Tekena Species) dried in dark environment was collected from Agricultural Development Agency (ADP), Ahoada Town in Rivers State of Nigeria while petroleum hydrocarbon was obtained from Eleme refinery, Rivers state if Nigeria. The loamy soil was tested and certified by Soil Science Department, Faculty of Agricultural in Rivers State University Port Harcourt and all samples were further transported to the Department of Chemical /Petrochemical Engineering Laboratory of Rivers State university Port-Harcourt for analysis. All necessary safety precaution was put on place while collecting all the samples to avoid contamination with other harmful substance.

Sample Treatment

Oil palm fibres were secured from the oil palm bunches of Tekena Species (improved) from Agricultural Development Programme (ADP) in Ahoada Town of Rivers State, herein refers as specimen A. The specimen A was treated in the presence of dark environment The sub-specimen were crush separately with a grader and sieved with particle size of 2.80mm taken and tie in a polythene labeled as follows; Specimen A - Improved fibres (Tekena Species) dried in dark environment

Samples Analysis

Elements Presence Using X-Ray Fluorescence Spectrometer

The elemental analysis was done using EDX3600B x-ray fluorescence spectrometer which applied XRF technology to conduct fast and accurate analysis of the sample. The system detects elements with atomic number 12 to 92. After pulverizing to homogenous size, it is calibrated using pure silver standard. Thereafter the working curve is selected using excel software and the output printed.

Type and Quantity of Micro-Organisms Presence Per Unit Gram

One gram of each sample was diluted and plated, on one plate in inoculated with nutrient agar (NA) incubated at 37 for 24hrs, while the other is inoculated with as bouraud dextrose agar (SDA) was incubated at ambient temperature for 3-5 days. After incubation counts on the ensuing colonies on the NA and SDA plates were used to calculate the bacterial and fungal population with the formula below,

$$Population\left(\frac{CFU}{ml}\right) = \frac{colony\ count}{volume\ plated\ x\ dilution\ plated} (3.0)$$

The bacterial isolates were subjected to microscopic examination and biochemical/physiochemical tests. Similarly, fungal colonies that developed on SDA plates were incubated at ambient temperature for 3-5 days, thereafter the fungal isolates were subjected to macroscopic and microscopic examination.

The Total Petroleum Hydrocarbon (TPH)

The pH was determined by measuring 2g of dry samples into 50ml beaker.20ml of diluted water was added and allows standing for 30 minutes and stirred occasionally with a glass rod. The electrode pH meter is inserted into the suspension and reading taken.

Conversely, the Total Petroleum Hydrocarbon (TPH) are gotten by weighing 2grams of the sample, then 10ml of extraction solvent (pentane). It is mixed, filtered and separated. The extracted samples were transferred to already prepare chromatographic

column. Concentrated Aliphatic fraction were transferred through a rubber septum separation occur and samples are detected as it emerges from the column by Flame Ionization Detector (FID) whose response is dependent upon the composition of the vapour.

In this Ex-situ remediation setup, seven reactors were used for this experiment. Each reactor were put 20kg of loamy soil. Six out of the seven reactors were polluted with 100ml of bonny light crude oil. Five out of the six polluted reactors were treated with 40kg of the various palm fibres biomass as demonstrated below.

Table 1 Demonstration of Experimental Set-up

S/No	Reactor	Descriptions	Mass of fibres
1	A	Polluted loamy + improve fibres (Dark)	40g

The reactor was kept in the chemical engineering laboratory (unit operation) with the covering removed to allow for oxygen and humility for the enzymes in the fibres to grow. 100ml of tap water was added to the reactors to maintain good moisture content for the microbes. Every five days the polluted samples mixed together to allow for even distribution of oxygen for effective remediation to take place. After two weeks the sample was taken to the laboratory for analysis for the P^H and Total Petroleum Hydrocarbon (TPH). Polluted soil sample was repeatedly analyzed for P^H and Total Petroleum Hydrocarbon (TPH) after every 14 days.

Kinetic Rate/Model Development

The Model Application

Kinetic Rate/Model Development

The local oil palm fibre dried in dark environment (F) which nurture the microorganisms and enzymes and the substrate (s) in the medium undergo the reaction represented by

$$[F] + [S] \underset{K_2}{\overset{K_1}{\rightleftharpoons}} [FS] \xrightarrow{K_P} [F] + [P] \qquad (1)$$

Assumptions

1.Enzymes formed react with substrate forming enzyme substrate complex.

2.The enzymes substrate decomposed to form product and enzymes.

3.The simple substrate controls the velocity of reaction.

The General Solution of Michalis Mentene Model

Recalling the Henry-Michael Menten equation which is expressed as

$$v = \frac{V_{max}(S)}{Ks + [S]} \qquad (2)$$

Where, Vmax = Maximum specific rate, V = Specific rate, Ks = Mcheelins content, S = Substrate (TPH)

Applying the law of conservation of mass on the reactor system, we have

$$\left[\text{Rate of flow of mass into the system}\right] = \left[\text{Rate of flow of mass out of the system}\right] - \left[\text{Rate of disappearance by chemical reaction}\right] + \left[\text{Rate of generation by chemical reaction}\right] + \left[\text{Rate of Accumulation within the system}\right] \quad (3$$

Assumptions

The reactor above is batch

There is no inflow and outflow of mass

There is no longitudinal and lateral flow of mass

There is uniform concentration as the medium is stirred before samples are taken for TPH analysis.
Hence;

Rate of inflow of mass = 0

Rate of outflow of mass = 0

Rate of formation = 0

Rate of disappearing = $-r_A V$

Rate of accumulation of mass = $\frac{dNA}{dt}$

Substituting into equation (3), we have

$$0 = 0 + (-r_A)V + \frac{dNA}{dt} \quad (4)$$

$$\frac{-dNA}{dt} = (-r_A)V$$

$$\frac{-1}{v}\frac{dNA}{dt} = -r_A$$

But $\frac{NA}{v}$ = concentration(s)

$$\frac{-dc}{dt} = \frac{-ds}{dt} = (-r_A)$$

But from equation (2), we have, $r_A = \frac{V_{max}[s]}{Ks+[s]}$

$$\frac{-ds}{dt} = -v = \frac{V_{max}[s]}{Ks+[s]} \quad (5)$$

Equation (5) is the kinetic of Michealis Menten model for determining rate of degradation of petroleum contaminants.
Where $\frac{-ds}{dt}$ = change in concentration of contaminant measure in TPH with time.

Method of Solution to Model

Due to the complexity of the monod rate equation developed, the Range – Kutta fourth order equation was used to obtain solution to rate equation. MATLAB computer program was used to solve the R – K equation and the algorithm is stated as follows:

$$S = f(t) \quad (6)$$

$$K_1 = hf\ (t(i), S(i)) \quad (7)$$

$$K_2 = hf\ (t(i) + \frac{h}{2}, s(i) + \frac{K1}{2} \quad (8)$$

$$K_3 = hf\ (t(i) + \frac{h}{2}, s(i) + \frac{K2}{2} \quad (9)$$

$$K_4 = hf(t(i) + h), s(i) + K_3 \quad (10)$$

$$S_{(i+i)} = S(i) + [k_1 + 2\ (k_2 + k_3) + K_4]\frac{n}{6} \quad (11)$$

Where h = step size

n = number of iteration

t = time

S = TPH concentration

K_1, k_2, k_3, k_4 = slopes

i = 1, 2, 3,

3. RESULTS AND DISCUSSIONS

In this investigation results obtained from the research work are presented in Tables and Figures as show

X-ray fluorescence spectrometer of Tekena Species

From the Chromatogram of Figure1the elemental composition is show in percentage as: potassium value of 2.3456, phosphorus value of 0.4398 and calcium value of 4.0510. This elemental value exceeds that of an organic matter in the ratio of 5:5:5 with respect to Nitrogen, Phosphorus and Potassium (NPK). This in terms of percentage is 0.33 and this implies that the species of oil palm fibre dried in dark environment is good for bioremediation of crude oil polluted site.

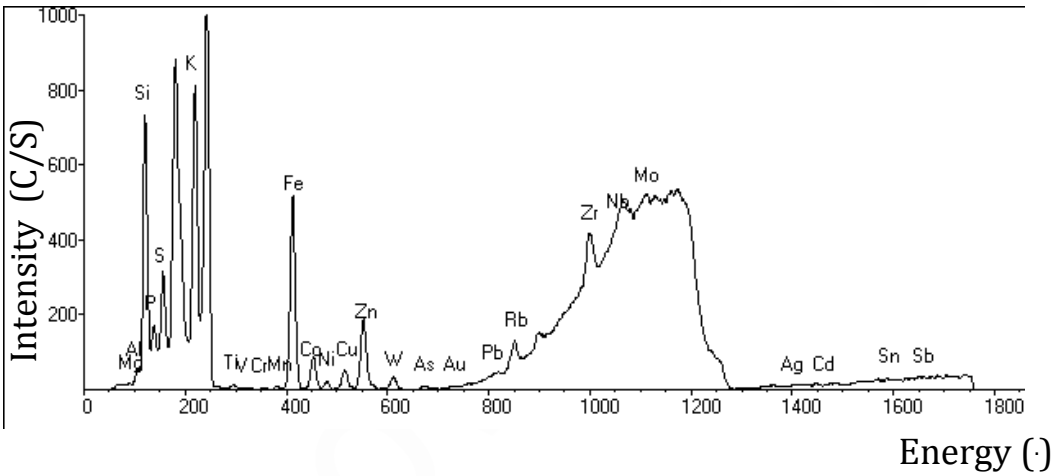

Figure 1 Chromatogram of Tekena Species

Types and Quantity of Microorganisms in *Tekena Species*

The *Tekena Species* has bacterial population of $9.60x10^6$cfu.g-1) while the fungal microbe has a colony forming unit of $1.27x10^5$ per gram. Hence, the total number of micro-organism in *Tekena Species* was high in population and capable of degradation of hydrocarbon contaminant. Identification of the Bacterial isolated from the *Tekena Species* reveals the bacteria present as *staphylococcus sp, Bacillus sp, Enterococcus sp, Lactobacillus sp,* and *corynebacterium sp* whereas the fungal isolated from the *Tekena Species* was grouped as *aspergillusnidulans, penicillum sp, verticilum sp, cladosporium sp, mucor sp,* and *conninhamella sp.*

Chromatogram profile for the TPH of unpolluted Loamy soil

From Figure 1 shows the amount of fractions of petroleum hydrocarbon present from n-C_8 to n-C_{40} the straight nature of this finger print indicate very small amount of hydrocarbon contaminant fractions in n – C_{23} to n – C_{34} other derivatives of petroleum Hydrocarbon percent has little or no negative effect on the soil as shown by the chromatogram.

Profile for the TPH of Polluted Loamy Soil

From Table 2 the chromatogram analysis results for the Petroleum derivatives from n-C_8 to n – C_{34} for various degradation periods is illustrated.

Table 2 Total Petroleum Hydrocarbons (TPH) of Polluted Loamy Soil + Improve Fibre (Dark)

COMPOUND NAME	DAY 0	DAY 14	DAY 28	DAY42	DAY 56	DAY 70	DAY 84
n – C8	51.29429	5.84567	51.29429	8.35842	8.35842	11.68342	2.03584 e^{-1}
n – C9	1.59863	2.01382	1.59863	1.31964 e^{-1}	1.31964 e^{-1}	1.542222	1.70534 e^{-1}
n – C10	3.24104 e^{-1}	1.81987	3.24104 e^{-1}	3.76383 e^{-1}	3.76383 e^{-1}	3.44138 e^{-1}	1.97993 e^{-1}
n – C11	5.64908 e^{-2}	0.84758	5.64908 e^{-2}	1.88560 e^{-1}	1.88560 e^{-1}	5.61521 e^{-2}	1.88760 e^{-1}
n – C12	2.60017	1.08973	2.60017	1.25660 e^{-1}	1.25660 e^{-1}	2.79044 e^{-1}	2.28427 e^{-1}
n – C13	4.38315	7.69427	4.38315	20.81098	20.81098	2.27232 e^{-1}	2.16711 e^{-1}
n – C14	14.20871	1.89720	14.20871	10.44368	10.44368	3.70461 e^{-1}	2.2155 e^{-1}
n – C15	5.43578	18.41675	5.43578	4.96533 e^{-1}	4.96533 e^{-1}	12.36533	2.82862 e^{-1}
n – C16	18.41675	52.40140	18.41675	16.26556	16.26556	1.89720	2.66393 e^{-1}
n – C17	52.40140	453.67444	52.40140	9.41350	9.41350	1.47330	8.36033 e^{-1}
Pristane	453.67444	54.99857	453.67444	8.31821 e^{-1}	8.31821 e^{-1}	193.48174	68.82419
n – C18	54.99857	656.15852	54.99857	11.86693	11.86693	5.49272	2.20447
phytane	656.15852	33.68592	656.15852	1.93715	1.93715	209.45719	2.8815
n – C19	33.68592	60.89942	33.68592	3.75928	3.75928	6.43809	3.98302
n – C20	60.89942	10.51848	60.89942	2.28923	2.28923	1.96893	3.24083 e^{-1}
n – C21	10.51848	5.22632	10.51848	3.89430	3.89430	4.58834 e^{-1}	5.6753
n – C22	4.24720	5.72387	4.24720	4.80744	4.80744	7.50211 e^{-2}	6.46551 e^{-1}
n – C23	42.75551	6.99995	42.75551	2.18482	2.18482	6.63571	7.7246 e^{-1}
n – C24	135.89143	3.88184	135.89143	2.17987	2.17987	58.8807	8.09889 e^{-1}
n – C25	8.46000	5.99028 e^{-1}	8.46000	2.38982	2.38982	6.06786 e^{-1}	1.23992
n – C26	8.80291	4.73529 e^{-1}	8.80291	4.21044	4.21044	1.30598	1.54019
n – C27	2.18632 e^{-1}	7.7540 e^{-1}	2.18632 e^{-1}	3.04070	3.04070	5.11472 e^{-1}	1.50916
n – C28	3.87470 e^{-1}	6.13130 e^{-2}	3.87470 e^{-1}	4.80960	4.80960	1.48965 e^{-1}	1.89946
n – C29	3.60934 e^{-1}	10.44368	3.60934 e^{-1}	2.72562	2.72562	1.12420	2.55108
n – C30	6.92624	4.96533 e^{-1}	6.92624	9.65279 e^{-1}	9.65279 e^{-1}	2.33295 e^{-1}	4.06557
n – C31	19.10384	16.26556	19.10384	3.69400	3.69400	5.84567	8.36221
n – C32	8.77871	9.41350	8.77871	2.37114	2.37114	2.01382	6.844545
n – C33	10.74807	3.75928	10.74807	2.63573	2.63573	7.85511 e^{-1}	8.87357
n – C34	1.62491	2.28923	1.62491	257.35808	257.35808	5.48587 e^{-1}	8.89803
n – C35	6.7206	3.89430	6.7206	4.27186	4.27186	7.511007 e^{-1}	13.80831
n – C36	11.95693	4.80744	11.95693	2.12927	2.12927	3.53753 e^{-1}	21.77987
n – C37	1.95874	2.18482	1.95874	246.00342	246.00342	1.86955	24.37141
n – C38	33.46704	2.17987	33.46704	5.22632	5.22632	1.69454	38.27722
n – C39	15.01115	2.38982	15.01115	5.72387	5.72387	4.10224	63.49936
n – C40	74.41567	1,3458	74.41567	6.99995	6.99995	9.25053	141.40061
$\sum(C_8\text{-}C_{40})$	1812.47426	1394.23	1023.69	847.18	698.74	544.25168	428.53522

Determination of Kinetic Constants

The maximum specific Rate constant and the Michealis Menten constant was determined from the line Waver Burk Plot for the biodegradable material investigated from this research as presented in Table 3.

Table 3 Data for Determination of Rate Constants for Improved Fiber (Dark)

Time (Day)	TPH (mg/l)	V (mg/l.day)	1/S (l/mg)	1/V (l.day/mg)
0	1812.47	31.56	0.00055	0.0317
14	1394.23	28.2	0.00063	0.0355
28	1023.69	19.53	0.00098	0.0512
42	847.18	11.61	0.00118	0.0861
56	698.74	10.82	0.00153	0.0924
70	544.25	9.65	0.00184	0.1036
84	428.54	6.88	0.00233	0.1453

Determination of Rate constant for improve fibre Dark

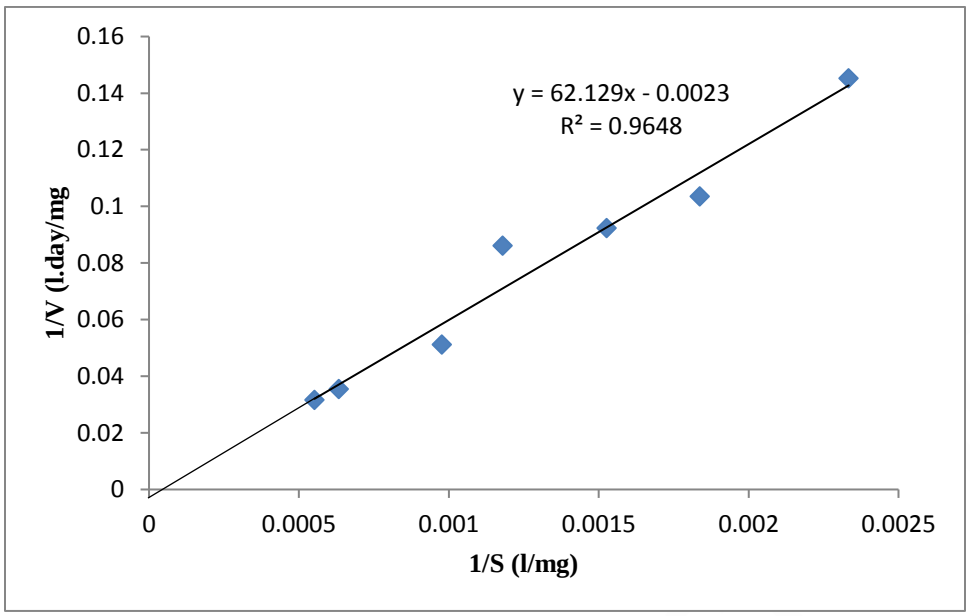

Figure 2 Line waver – Burke Plot for improved fibre Dark

Figure 2 shows the Line Waver-Burke Plot for the determination the kinetic rate constants using improved fibre treatment in dark environment. The equation of the line is given as y = 62.129x-0.0023 with the square of the best fit given R^2=0.948. The maximum specific rate was 434.78 mg/l. day while the Michaelis rate constant was 27912.61mg/l. Therefore, the kinetic rate model describing the biodegradation of TPH in polluted soil under the influence of improved fibre in dark environment, according to equation (5) can be expressed as

$$-\frac{dS}{dt} = -V = \frac{434.78[S]}{27012.61 + [S]}.$$

Model and Experimental Performance for Improved Fibre Dark

The TPH concentration is plotted against time shows steady decrease in contaminants.

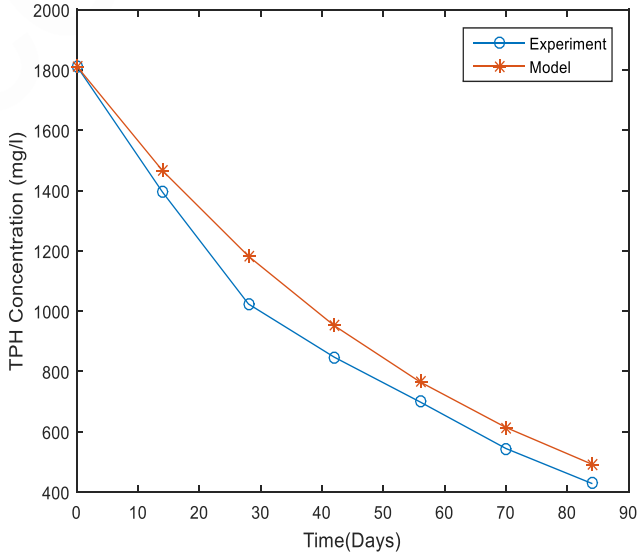

Figure 3 Performance of Improved Fibers (Dark) in TPH Reduction in Soil for Model and Experiment

The performance of improve fibre Dark in degrading the petroleum hydrocarbon out the concentration of 1812.47 mg/L at day zero for both model and experiment. Both plots show decrease in concentration of TPH. At 42days investigation, the model degraded 60.709 mg/L of contaminant while the biomass degraded 965.29mg/L. At the 84 days investigation the contaminant remaining for the model is 492.417 mg/L while that for experiment remains 428.54mg/L. Hence the percentage degradation for model is 72.8%.

4. CONCLUSION

Bioremediation of crude oil polluted loamy soil using oil palm fibre is proven to be very effective in restoring the soil to its original status. Palm fruit fibres of *Tekena Species* have an NPK values well enough for remediation. Palm fruit fibre species treated at various conditions degraded the petroleum Hydrocarbon at different rate by measuring the total petroleum Hydrocarbon (TPH). The data generated by the model are similar to the data gotten by the experiment, hence validating the model. Performance evaluation of the improved oil palm fibre in remediating contaminated is a welcome development in the field of engineering and environmental sciences.

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