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Degradation biokinetics of used and fresh lube oils in contaminated soil environment

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A B S T R A C T

In order to solve the problems of the soil environment caused by the discharge of petroleum products especially oils used for lubrication, investigations were carried out to assess the degradation of fresh and used lube oil in soil environment using bacteria degraders present in the soil. This was done by introducing some quantities of the pollutant (fresh and used lubricating oils) into some soil samples obtained from the environment. The degradation pattern was studied by estimating the level of the microbial growth using culture method and the phenol content. This was done before the introduction of the pollutant and at two weekly intervals for 42days. Results show that the phenol content, microbial and micro species present in the soil rapidly increased to maximum and then declined. The specific rate, maximum specific rate and equilibrium constants were determined using Line-Weaver Burk Plots. Results obtained show that the bacteria which degraded the fresh and used lube oil were inhibited by the presence of phenol which was informed during the biodegradation reaction. Empirical equation for microbial growth rate using the data from the experiment was obtained. The degradation of the fresh lube oil occurred faster than the used lube oil. The equation is useful in monitoring and predicting the performance of microorganisms in bioreactor and predicting the performance of microorganisms in bioreactor containing used or fresh lube oil.

Capsule Summary: Degradation pattern of fresh and used lubricating oil in soil samples was studied by estimating the microbial growth through modeling. The model was useful in monitoring and predicting the performance of microorganisms in bioreactor containing used or fresh lube oil.

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INTRODUCTION

The potential environmental pollution impact from petroleum operations has been a matter of concern to environmentalist. This is common in Nigeria, particularly in the Niger Delta region. The various governmental and non-governmental agencies have been actively involved in trying to check the excesses in the petroleum industry. On the part of the multinationals involved, government has been encouraging them to treat their waste before discharging into the environment. In response to this, various

treatment/waste disposal techniques are being used to achieve these objectives. Such techniques include preliminary treatment is classified as chemical and biological processes. Bioremediation which is a biological process has of late gained tremendous prominence (Ritch, 1973; Riggs et al., 1970; Miller and Alexander, 1991; Kamanayalli and Ninnekar, 2004; Jadulco et al., 2004).

In addition to high quality fuel, lubricant materials are also essential for normal operations of various engines and mechanisms. All lubricants can be divided into four types: gaseous, solid, liquid and semi-solid (thickness) or grease; petroleum processing industry manufactures mineral oils of many kind: motor oils (aviation diesel and automobiles grades), industrial oils, turbines oils, electro insulting oils, compressor oils etc; Viscosity is the most important characteristic of all kinds of oil. Other important characteristics of oils are as follows: (i) oxidation stability, (ii) low setting point, (iii) good anticorrosive properties and others. All grades of oils for modern mechanism and engines especially for diesel engines contain additives which improve their performances (Peter et al., 2003; O'Connor and Dobbins 1958; Oh et al., 1994; Mulkins and Stewards, 1974; Metcalf and Eddy, 1991; KWW, 2001)). In industrial practice, standard hydrocarbon type for internal combusting engines lubricating oil, a typical base oil stock, may contain about 64-68% paraffin, 28-33 percent nephthenes and less than 1 percent aromatic hydrocarbon (Abbey et al., 2003; Chaloupkova et al., 2003; Carey et al., 2003; Bradley and Chapelle, 1996; Antai, 1992)). Petroleum base lubricating oils are presented in the residue above 370^{o}C (680^{o}F) from the atmospheric distillation of selected oils of both paraffin and Naphethenic types. Finished lubricating oils are then made by blending these refined stocks to a desired viscosity followed by introducing additives needed for optimal performance. In Nigeria make use of oil base from Kaduna Refinery using foreign crude oil from Kuwait, Venezuela among others. Lube oil blending plants in Nigeria make use of base oils from Kaduna lube oil refinery or imported base oil (with low additives to get the finished products of various grades for different applications. Management of the used oil in United States (Environmental Regulation and Technology 1994) shows that used lube oil generated by automotive and industrial generators is either dropped or used or used in site or gathered by collectors, who then act as suppliers to reclaiming facilities or oil dealers. Presently, in Nigeria, there is no recycling facility where the used lube oil is processed for better utilization rather they are disposed in manners that pollute the environment (Dauda and Obi, 2000; De-Wideman et al., 2004; Dercova et al., 2003; Dror and Schlautaman, 2004; Dyer, 2003).

Microorganisms are microscopic living creatures that are widely found in the biosphere. These microbes interact with other living organism and are responsible directly or indirectly for some of the major events in their day to day activities. Investigation into this microorganism reveals that, their metabolic activity are very complex and efficient, with the heat and mass transfer operations obeying the physical and chemical laws (Goldsmith and Balderson, 1989; GEMS 1992; Fuse et al., 2003; Feng, 2004; Evans, 1963; Germ et al., 2001). Industrial microorganism from biochemical perspective is an aspect of socio-chemical engineering that deals with study of microorganisms and their uses in process and environmental engineering. The use of microbes in tackling environmental pollution and has enhanced the development of new area of environmental technology such as bioremediation of lube oil, contaminated environment; this research work is therefore aimed at determining and investigating the rate of biodegradation of both fresh and used lubricating oil in the soil with a view to providing data for the development of bioremediation programme for lube oil in the soil (Holliger and Zehnder, 1996; Haggblom et al., 2003; Hong-gyu and Richard, 1990; Ilsam, 1990; Jadulco et al., 2004).

According to National database of petroleum products consumption, the total domestic consumption of lubricating oils in 1996 was 304, 106, 000 liters. Unfortunately, there is no record of how the used lube oil was managed. This could be attributed to unstructured nature of disposal and lack of governmental regulation requiring dissemination of the data on used oil collection, handling and disposal practices. By indication it is careless to dispose used lube oil thereby contaminating the environment soil (Mravik et al., 2003; Bugni and Ireland, 2004; Blunt et al., 2004; Bailey and Ollis, 1986). Fresh and used lube oil is a potentially hazardous material and improper disposal is capable of containing soil, underground and surface water and will endanger the public health; as a result of the potential environmental pollution that the indiscriminate dumping of petroleum products, especially used lubricating oil has caused, therefore there is need for Engineers, Environmentalist and other research groups and individuals to direct their efforts at solving the associated environmental problems; hence, this research work is aimed at investigating the rate of biodegradation of fresh and used lube oil in the soil in view of providing data for bioremediation programme for the effective bioremediation of lube oil in the soil (Ahmed et al., 2003; Ahmed, 2004; Abdel-Salam and El-Shafi, 2004; UKpaka 2014, 2014a, 2014b, 2014c; Ukpaka 2015, 2015a, 2015b, 2015c, 2015d, 2015e).

To determine the physiochemical properties of fresh and used lube oil; to determine the rates of biodegradation of fresh and used lube oils using microbial growth rate plots; to develop polynomial expression for the biodegradation of lube oil in the soil.

Adequate treatment whereby microorganisms are injected into the waste before disposal of used lubricating oil sewers/environment is very necessary as this will check the destruction of the soil environment.

The development of bioremediation programme for the effective bioremediation of lube oil in the soil is very important in view of the dangers posed to the soil fertility which is necessary for sustenance of agriculture. Therefore, research works such as this would provide valuable data for assessment of the rates of biodegradation of petroleum

products, including used and unused lube oils that are indiscriminately dumped in the environment. Results from such studies would enable us put in place effective bioremediation measures to mitigate the adverse impact on the environments

The scope of this research work shall focus on the experimental and theoretical investigation of the fresh and used lube oil degradation in the soil environment. The experimental investigation shall cover the enumeration of hydrocarbon degraders in the soil contaminated with fresh and used lube oil which shall involve microbial culturing and estimation of the phenol concentration using the Bench Method while the theoretical investigation shall be centered on the calculation of the specific rate, maximum specific rate and equilibrium constants. A polynomial expression for the biodegradation of lube oil soil is also to be developed.

MATERIAL AND METHODS

The section is subdivided into two: Theoretical and Experimental methods

Theoretical and calculation methods

Theoretical kinetic model: Based on Henri model the lube oil degradation reaction can be represented as follows:

$$[L]+[M] \underset{K_2}{\overset{K_1}{\rightleftarrows}} [LM] \overset{K_3}{\rightarrow} [M]+[P] \quad (1)$$

The rate of the reaction can be expressed as

$$R = \frac{d[P]}{dt}$$

$$R = \frac{-d[L]}{dt} \quad (2)$$

The rate could be expressed as

$$R = \frac{K[L]}{1+\frac{[L]}{K_L}} \quad (3)$$

Where [L] = the lube oil (substrate concentration)

[K_L] = the dissociation constant of the complex and is equal to

$$K_2 / K_1 = \frac{[M][L]}{[LM]} \quad (4)$$

K_3 = rate constant for the breakdown of $[LM] \rightarrow [M]+[P]$

K = a constant characteristics of the particular microbes preparation.

$$K = \left[\frac{K_3[M]L}{K_L}\right] \quad (5)$$

At any given time the total number of microbes M_t can be defined as

$$[M_t] = [M]+[LM] \quad (6)$$

Where, [M] represent number of free microorganism. Since the rate of reaction depends on the dissolution of the microbes substrate complex, the product forming complex.

Then,

$$R = K_3[LM] \quad (7)$$

Now combining equation (6) and (7) yields

$$\frac{R}{[M_t]} = \frac{K_3[LM]}{M+[LM]} \quad (8)$$

Expressing the concentration of each microbial species in terms of free microbe gives

$$[LM] = \frac{[L][M]}{K_L} \quad (9)$$

Substituting equation (9) into equation (8) yields

$$\frac{R}{[Mt]} = \frac{K_3\frac{[L]}{K}[M]}{[M]+\frac{[L]}{K_L}[M]}$$

$$\frac{R}{K_3[Mt]} = \frac{\frac{[L]}{K_L}}{1+\frac{[L]}{K_L}}$$

$$\frac{R}{K_3[Mt]} = \frac{[L]}{K_L\left[1+\frac{[L]}{K_L}\right]}$$

$$\frac{R}{K_3[Mt]} = \frac{[L]}{K_L+[L]} \quad (10)$$

The maximum rate of reaction can be expressed as:

$$R_{max} = K_3[Mt] \quad (11)$$

Substituting equation (11) into equation (10)

$$\frac{R}{R_{MAX}} = \frac{[L]}{K_L + [L]} \quad (12)$$

Equation (12) is in the form of Henri-Michaels Menten equation for a single substrate any single microbe

$$\frac{R}{R_{MAX}} = \frac{[L]}{K_L + [L]} \quad (13)$$

Simplifying equation 3:13 gives

$$R = \frac{R_{max}[L]}{K_L + [L]} \quad (14)$$

Equation (14) gives the rate of biodegradation of lube oil under the condition stated above considering lube oil as a single substrate. The kinetic data in the model can be evaluated by taking the reciprocal of equation (14) and rearrangement to put in the term of a linear equation.

$$\frac{1}{R} = \frac{[L] + K_L}{R_{max}[L]} = \frac{1}{R_{max}} + \frac{K_L}{R_{max}[L]}$$

$$\therefore \frac{1}{R} = \frac{1}{R_{max}} + \frac{K_L}{R_{max}[L]} \quad (15)$$

This technique known as Line-Weaver Burk plot is widely used for the determination of the kinetic data required to evaluate the specific reaction. A Line-Weaver Burk plot shall be made for the lube oil in order to determine the R, K_L and R_{max} in the study.

Calculation of kinetic parameters: Data generated from the experimental readings are used to plot the Line-Weaver Plot. In order to calculate the maximum specific rate, R_{max} and the maximum rate constants K_L in the Line-Weaver Burk Equation (15), the equation of best fit from graphs of specific rate 1/R versus substrate 1/L is used. Line-Weaver Burk equation is of a straight line Equation,

$$Y = mX + C, \quad (16)$$

Where, the Y-axis is (1/R); the X-axis, is (1/L) and the slope of the line m represents K_L/R_{max} and C = $1/R_{max}$

The values for the kinetic parameters are easily evaluated from the intercepts on both axes. The intercept on Y-axis gives the value for maximum specific rate, while the reciprocal of the intercept on the X-axis gives the value equilibrium rate constant (K_L).

Experimentation

Two different experiments were carried out with the samples prepared:

i. Enumeration of the hydrocarbon degraders in the various soils and

ii. The estimation of the phenol content.

Materials used

The following materials were used for the investigation: fertile loamy soil, seven (7) plastic buckets, sterile sample bags, fresh lubricating oil, and used lubricating oil.

Equipment's

Spectrophotometer, filter funnels, filter papers, pH meter, separating funnels (1 litre)

Reagents

All reagents were prepared with distilled water.

i. The minimum salt medium for the enumeration of hydrocarbon degraders was prepared as described by Mulkins-Philip and Stewart 1974. This was mixed with Agar-agar, lube oil, carbon tetrachloride and then sterilized. 10gm of the soil sample was taken and diluted serially into 10, 10^2 and 10^3. 1ml was taken and poured on the surface of the agar medium prepared. To enhance bacteria growth and suppress fungi, 0.003% phenol red was added.

Bacteria plate were incubated upside down at 35 ± 0.8 ^{0}C.

ii. Phenols react with 4-amino antipyrine at pH range of 7.9-10.2 ± 0.1 in the presence of potassium ferricyanide to form a coloured antipyrine dye. This dye is extracted from aqueous solution with $CHCl_3$ and the absorbance is measured at 460mm. This method covers phenol concentration range from $^{1.0\mu g/L}$ with a sensitivity of $^{1.0\mu g/L}$.

Samples collection and preparation

Mode of collection: A portion of fertile parcel of land within the Petroleum Training Institute (P.T.I) Effurun in Delta State of Nigeria was cleared and soil was excavated from the land. The soil was a loamy soil. Five kilograms (5kg) of soil was put each of the seven plastic buckets as samples. Soil samples were also collected for analysis and characterization during the raining seasons. The soil samples were also collected from the location sites at a distance randomly taken by means of a soil augur at a depth of 015cm. the soil samples were thoroughly mixed so as to obtain a homogenous representation. The composite soils taken represent the true status of soil characteristics in the area.

Preparation of samples for analysis: Used lube oil from the generator house as well as fresh lube oil of the same characteristics were collected. The soil samples in the plastic buckets were then polluted with different quantities of both fresh and used oils separately. These quantities were measured with measuring cylinder. Each was separately well mixed and left for the period of the experiment. Portions of

the samples were each time transported into the laboratory for analysis.

The first (1st) container was labeled as the control as the soil was not polluted (contaminated) with any of the lubricating oils.

i. The soil in the second (2nd) container was polluted with 10 ml of fresh lube oil.

ii. The soil in the third (3rd) container was polluted with 20 ml of fresh lube oil.

iii. The soil in the fourth (4th) container was polluted with 30 ml of fresh lube oil.

iv. The soil in the fifth (5th) container was polluted with 10 ml of used lube oil.

v. The soil in the sixth (6th) container was polluted with 20 ml of used lube oil.

vi. The soil in the seventh (7th) container was polluted with 30 ml of used lube oil.

The initial level of the hydrocarbon degraders and the phenol content of the unpolluted soil sample were enumerated and estimated respectively. The different preparations were well mixed and allowed to remain for two (2) weeks. After the two weeks, samples were taken from each of the labeled containers and put in sterilized bags obtained from a microbiology laboratory and sent for culturing. A third sampling was made after a further two weeks (6wks) and laboratory analysis for the estimations of the hydrocarbon degraders and phenol concentrations made for each of the period.

Microbial culture and enumeration of hydrocarbon degraders

These experiments are aimed at studying the effect of the pollution on the microorganisms (degraders) present in the soil. The methods of Buchana and Gibbons (1974) and Gerbardt et al. (1981) were applied for the determination of the Microbial population.The seven main experimental units were set- up to generate the required data for the investigation.

The population of the microorganisms present in the system which is capable of degrading the fresh and used lube oil was determined by counting the colony forming unit/gram (cfu/g) in a pour plate of mineral salt agar medium containing 0.5% of the preparation of the soil reagents made in paragraph 2.1.3 for each fresh and used lube oils samples.

The plates were incubated upside down at 35± 0.8°C. Microbial growth was observed after the period of incubation. Colonies were counted and computed as described in standard plate counts.

Phenol concentration (Bench method)

Chemical analysis was made to estimate the phenol concentration in each sample. Phenol is a toxic byproduct of the degradation of lube oil. The procedure adopted is as follows:

Soil sample (5 g) was dissolved in 30ml of distilled water. The mixture was placed on a magnetic stirrer for 5 minutes, and then filtered out. 10ml of the extract was diluted to 300ml and used for the analysis using the spectrophotometer.

Data processing

Line-Weaver Plots were used to process data collected and to evaluate R_{max} and K_L as shown in section 2.1.2. Polynomial curve fitting technique was also used to describe the microbial growth.

Basic assumptions using phenol as substrate

At early stages of degradation we assume that all microbial action to degrade the lube oil results in the formation of phenol and if we equate the initial rate of biodegradation of lube oil to the rate of phenol formation, we can then directly estimate the specific rates of the lube oil degradation rate constants using changes in phenol concentration as an indicator of changes in substrate, L.

Thus, in Line-Weaver Burk plot we replaced the substrate concentration, L with the concentration of phenol in equation (16).

RESULTS AND DISCUSSION

The results obtained from the various experiments are presented in Tables and Figures as shown below.

Characteristics of soil samples

Table 1 shows that the soil samples is sandy and slightly acidic with pH of 6.5, rich in calcium content (14.95mg/100gm of soil) as opposed to low content of sodium (Na), magnesium (Mg) and potassium (K) whose concentrations are 1.17, 1.22 and 1.65 mg/100gm of soil respectively. Total hydrocarbon in the soil samples is 4.54μ/g.

Hydrocarbon degraders in fresh and used lube oils

The results in Tables (2) and (3) cover data for a period of 42 days of an uninterrupted sampling and analysis. Monitoring of changes in phenol concentration and microbial counts for hydrocarbon degraders was meticulously followed.

Generally the hydrocarbon degraders increased rapidly to maximum values and declined thereafter. The peak value for hydrocarbon degraders was at the 28th day analysis for all samples-both for the fresh and used lube oil.

Phenol concentration profile in polluted soil

The phenol concentration also followed the same trend as those of the hydrocarbon degraders and attained a peak on the 14th day of contamination then declined to a minimum with further increase in numbers of days, which indicates that the biokinetics degradation of used and fresh lube oils in contaminated soil environment was significant.

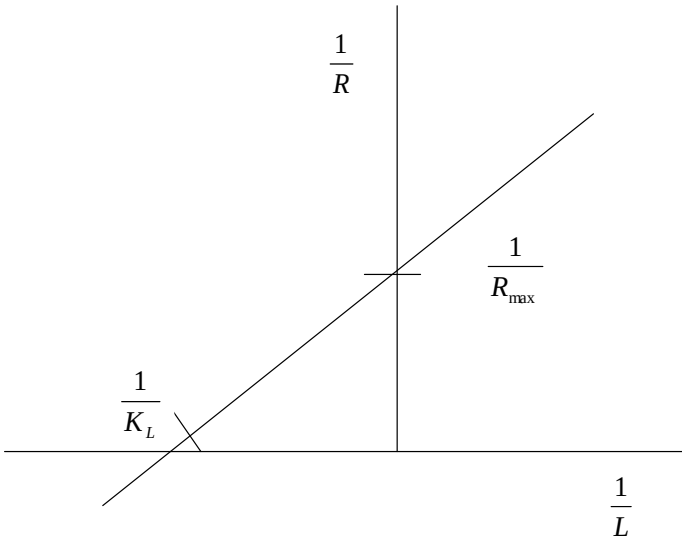

Fig. 1: Line-Weaver Burk plot

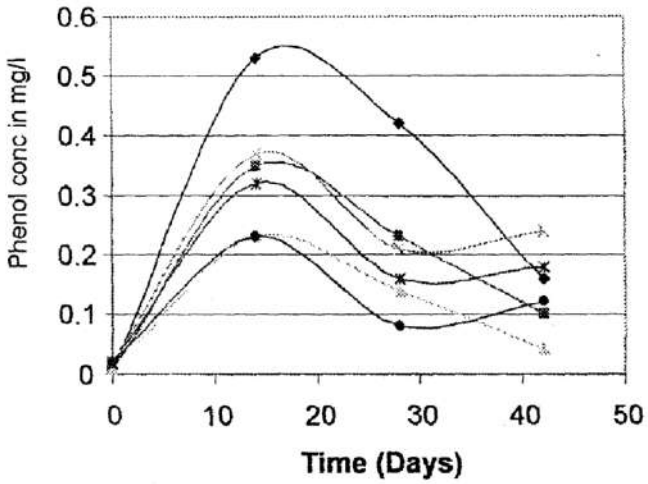

Fig. 2a: Graph of phenol concentration in soil polluted with different volume of lube oil against time from day zero.

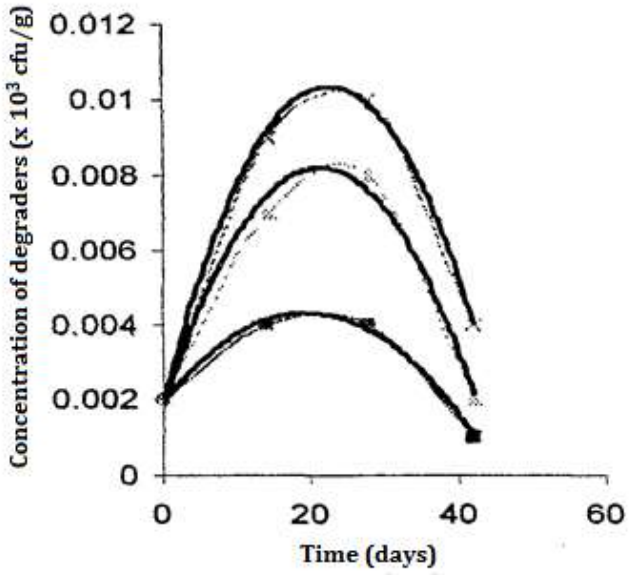

Fig. 2b: Concentration of hydrocarbon degraders in fresh lube oil against time in days

Evaluation of R_{max} and K_L

Using phenol as a substrate from Table 3.3 the following theoretical calculations were made:

Calculations:

$$(L)_o^F \quad 0.01; (L)^U{}_O = 0.02$$

Where $(L)_O{}^F{}_M$ = Initial phenol concentration in fresh oil and

$(L)^U{}_O$ = Initial phenol concentration is used oil

For substrate (L), take values as in Table 2

For specific Rate,

$$R = (L)/t = (L)_2 - (L)_1 / t_2 - t_1$$

For fresh 10 ml

Substrate concentration:

Final-Initial concentration, 0.53-0.01 = 0.52 ml/l

Therefore Specific Substrate concentration = 1/L which is = 1/0.52 =1.92

Specific Rate, R = 0.52/14 =0.037. Therefore 1/R = 1/0.037 = 27.02

This was done for the various concentrations and the results obtained are computed as shown in Tables 4 to 7.

The data obtained in Table 3 i.e. the concentration of phenol for the different levels of pollution were plotted against the time of the experiment and Figures 1 was obtained for fresh and used lube oil.

In this work phenol was used as the substrate as in the second experiment because it is known that hydrocarbons when oxidized, produce phenol as a by-product. It is known that the oxidation at the point of application is accompanied with the release of free radicals that transform to peroxides, which subsequently condensate and polymerize and produce per-acids, naphthenic acids etc. It is also widely agreed that toxicity of petroleum hydrocarbons which includes lube oil rises with increase in molecular weight, total acid number and presence of cyclic hetero-atoms and substitutes.

The toxicity of phenol together with that produced as a result of the decomposition of the incorporated additives in the lube oil aided the fast degradation of the biomass after, especially in the case of the used lube oil.

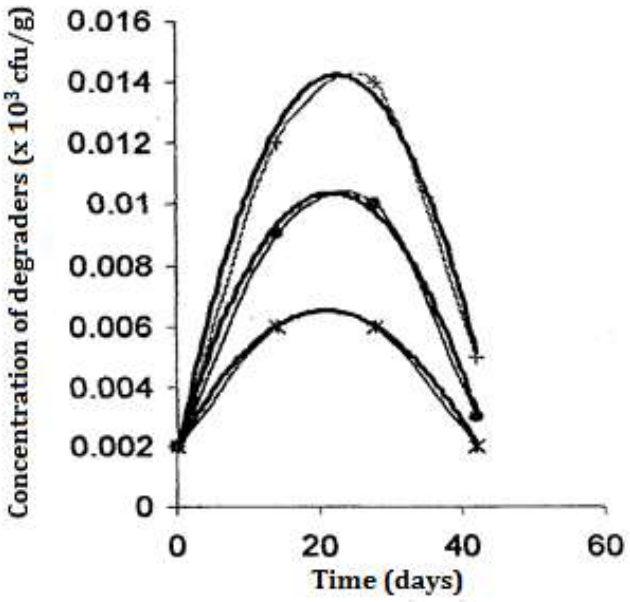

Fig. 3: Concentration of hydrocarbon degraders in used Lube Oil against Time (days)

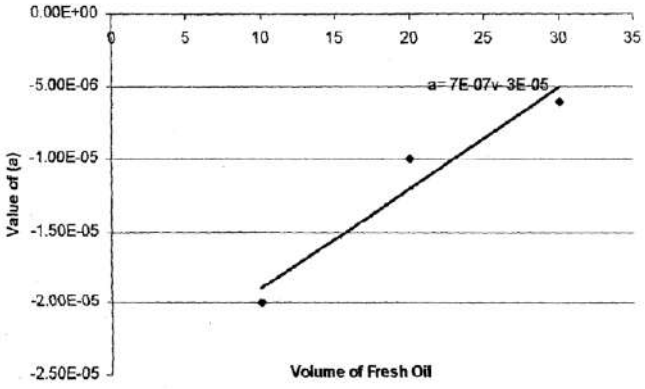

Fig. 4: Value of (a) against volume of fresh oil in soil

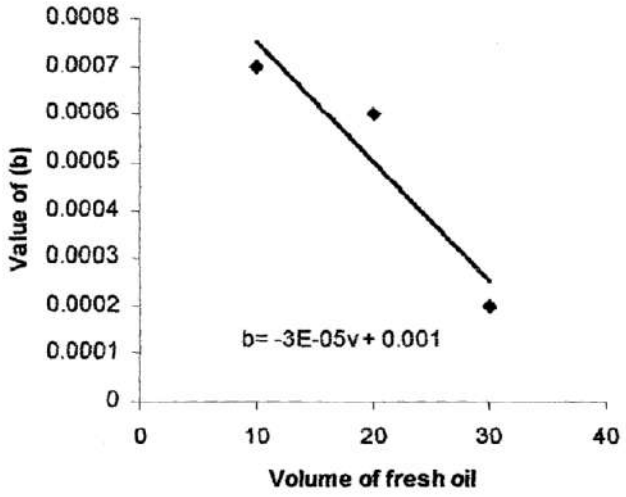

Fig. 5: Value of (b) against volume of fresh oil in soil

The level of phenol was much higher in used lube oil than in fresh lube oil probably because under usage, the lube oil partly underwent oxidation reaction, which generated alcoholic hydrocarbons. Generally it is however known that lube oils have low water solubility but as a result of the presence of microorganisms in the soil environment, coupled with the additional constituents in the used lube oil the oils were degraded faster than fresh lube oil. Results in Figure 2a and 3 shows the variation of concentration of hydrocarbon degraders with time for both fresh oil (Fig. 2a) and used lube oil (Fig. 3). The plots show an initial rapid increase in the buildup of hydrocarbon degraders which is followed by a decline after 20 days. The degradation in used lube oil was however discovered to be faster probably because of metallic components from shear/wears and incomplete combustion of the lube oil in the engines. The decrease in the microbial growth rate generally is proportional to the microbial concentration and the presence of toxic species as seen after the 14th day when death of the microbes began to be experienced. The results as obtained from the computation using the formulated models had similar patterns with those determined from the experimental analytical results.

Rate of microbial growth

Results in Figure (2a and 3) for hydrocarbon degraders show that the growth of the microbes is a polynomial expression of the form of a quadratic equation.

$$M = at^2 + bt + c \qquad (16)$$

Where, a and b are numerical constant and c is the initial microbe content in the soil.

M stands for microbes' population and t stands for reaction time.

Depending on the degree of soil pollution, individual equations for the population of microbes are as follows:

For fresh oil polluted environment

$M = 2E\text{-}05t^2 + 0.0007\,t + 0.002$ (10 ml) (17)

$M = -1E\text{-}05t^2 + 0.0006t\ 0.0019$ (20 ml) (18)

$M = -2E\text{-}05t^2 + 0.0002t + 0.002$ (30 ml) (19)

For used oil polluted environment

$M = -1E\text{-}05t^2 + 0.004t + 0.002$ (for 10 ml) (20)

$M = -2E - 05t^2 + 0.008t + 0.001$ (for 20 ml) (21)

$M = -2E - 05t^2 + 0.0011t + 0.0017$ (for 30 ml) (22)

These equations show that there is an initial rapid buildup of microbes, which later stabilized and then decayed.

From these equations we can predict the time for total disappearance of the hydrocarbon degraders. This time will correspond to total inhibition of degradation of the lube oil by the phenol formed at the early stages. This occured when M = c in equation (1) to give t = 0 or (-b/a) days.

Table 1: Physicochemical parameters of soil samples

Parameters	Units	Concentration
pH	-	6.5
Organic carbon	%	1.81
Total nitrogen	%	4.24
Total hydrocarbon (THC)	µg/g	4.54
Available phosphorous	µg/g	4.54
Potassium	mg/100g soil	1.65
Sodium	mg /100g soil	1.17
Magnesium	mg /100g soil	1.22
Calcium	mg /100g soil	14.95
Sand	%	58
Silt	%	16
Clay	%	20
Hydrocarbon Degraders	X 10^3 cfu/g	0.002

Table 2: Concentration of hydrocarbon degraders for fresh and used lube oil in soil environment (X 10^3 cfu/g)

		Concentration of soil polluted with fresh and used lube oil (g/ml)					
Time (day)	Control	10 ml (fresh)	20ml (fresh)	30 ml (fresh)	10 ml used)	20 ml (used)	30 ml (used)
0	0.002	0.002	0.002	0.002	0.002	0.002	0.002
14	0.0002	0.004	0.007	0.009	0.006	0.009	0.012
28	0.0002	0.004	0.008	0.010	0.006	0.010	0.014
42	0.0002	0.001	0.002	0.004	0.002	0.003	0.005

Table 3: Analysis of Phenol Concentration (L) in soil polluted with fresh and used Lube oil

	Concentration of soil polluted with fresh and used lube oil (g/ml)					
Time (day)	10 ml (fresh)	20ml (fresh)	30 ml (fresh)	10 ml used)	20 ml (used)	30 ml (used)
0	0.01	0.01	0.02	0.02	0.02	0.02
14	0.53	0.35	0.23	0.37	0.32	0.23
28	0.42	0.23	0.14	0.21	0.16	0.08
42	0.16	0.10	0.04	0.24	0.18	0.12

Table 4: Computed specific substrate (phenol) concentration in fresh lube oil contaminated soil

Time (day)	$(L)_{10}^{F}$	$(L)_{20}^{F}$	$(L)_{30}^{F}$	$1/(L)_{10}^{F}$	$1/(L)_{20}^{F}$	$1/(L)_{30}^{F}$
14	0.52	0.34	0.22	1.92	2.94	4.55
28	0.41	0.22	0.13	2.44	4.55	7.69
42	0.15	0.09	0.03	6.67	11.11	33.33

Table 5: Computed rates and specific rates of substrate degradation for fresh lube oil polluted soil

Time (day)	$(R)_{10}^{F}$	$(R)_{20}^{F}$	$(R)_{30}^{F}$	$1/(R)_{10}^{F}$	$1/(R)_{20}^{F}$	$1/(R)_{30}^{F}$
14	0.037	0.024	0.016	27.03	41.67	62.5
28	0.015	0.008	0.0046	66.7	125	217.39
42	0.0036	0.002	0.0007	277.8	500	1428.57

Table 6: Computed specific substrate (phenol) concentration for used lube oil contaminated soil

Time (day)	$(L)_{10}^{U}$	$(L)_{20}^{U}$	$(L)_{30}^{U}$	$1/(L)_{10}^{U}$	$1/(L)_{20}^{U}$	$1/(L)_{30}^{U}$
14	0.35	0.30	0.21	2.88	3.33	4.75
28	0.19	0.14	0.14	5.26	7.14	16.67
42	0.22	0.16	0.16	4.55	6.25	10.0

Table 7: Computed rates and specific rate of substrate degradation for used lube oil polluted soil

Time (day)	$(R)_{10}^{U}$	$(R)_{20}^{U}$	$(R)_{30}^{U}$	$1/(R)_{10}^{U}$	$1/(R)_{20}^{U}$	$1/(R)_{30}^{U}$
14	0.025	0.021	0.15	40	47.62	6.67
28	0.007	0.005	0.002	142.86	200	500
42	0.005	0.004	0.0024	200	250	416.7

The time, t, in equations (17) to (20) at same condition when M = c can be solved, using values of a, b. The following results were obtained, as shown in Table 4 below.

The relationship between a, b and V can be generated by plotting the values for a and b against V (volume of fresh oil) to give graphs in Figures 4 and 5 respectively, whose equations are:

$$a = 7E-07V-3E-05 \; and \; b = 3E-05V+0.001$$

Thus a generalized form of equation 4.1 for soils polluted with fresh oil will become;

$$M = (7E-07V-3E-05)\,t^{2+}(-3E-05+0.001)\,t+C \quad (23)$$

$$M_F = (0.7V-3)X10^{-5}t^2+(1-0.03V)X10^{-3}t+C_F$$

And similarly for used lube oil,

$$a_u = (-7-05V)X10^{-6} \; and \; b_u = (7.3-0.1V)X10^{-3}$$

$$M_U = -(-7-05V)X10^{-6}t^2+(7.3-0.1V)X10^{-3}t+C_U$$

Values of kinetic parameters

The maximum specific rate, R_{max} and the maximum rate constants K_L in the Line-Weaver Burk Equation, which were obtained using the equation of best fit from graphs of specific rate 1/R versus reciprocal of substrate 1/L. From values in Tables 5 to 8, the various plots obtained are shown in the Figures (6 to 10) below.

R_{max} and K_L for fresh oil polluted soil

For pollution with 10ml, Figure 3.6 illustrates the graph of $1/R_{10}^{F}$ and it is seen that the specific rate of fresh lube oil increases with decrease in the reciprocal of the substrate. The graph obeys the Line-Weaver Burk Plot.

Therefore the maximum specific rate, the equilibrium constant (K_{L10}^{F}) and the R_{MAX} can be evaluated mathematically as;

$$Y_{10} = 51.681x-66.169$$

$$R_{max} = -1/66.169 = -0.015;$$

$$K_L/R_{max} = 51.681,$$

$$K_L = -0.78$$

For pollution with 20ml, figure (7) illustrates the graph of $1/R_{20\text{-}F}$ and it is seen that the specific rate of fresh lube oil increases with decrease in the reciprocal of the substrate.

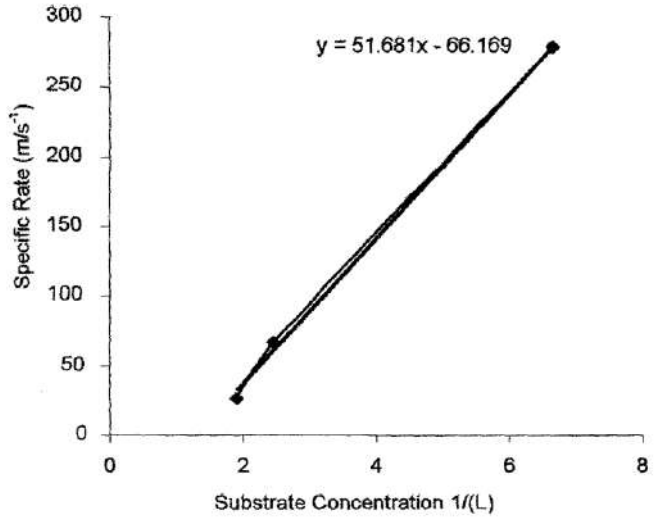

Fig. 6: Graph of Line-weaver Burk Plot for substrate concentration against specific rate for 10 ml of fresh Oil

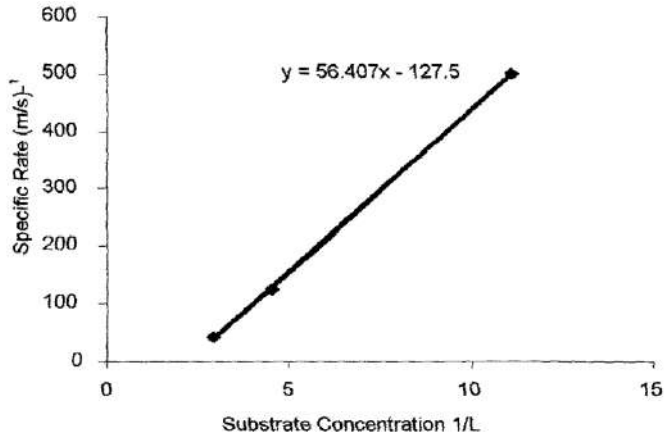

Fig. 7: Graph of Line Weaver Burk Plot for 20 ml of fresh lube oil

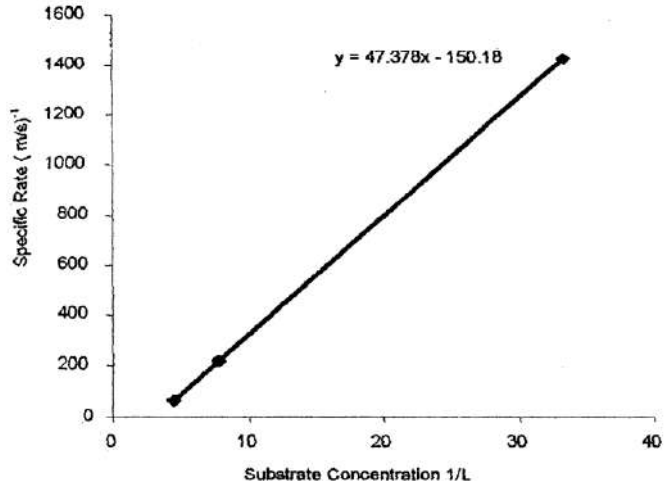

Fig. 8: Graph of Line Weaver Burk for 30 ml of fresh lube oil

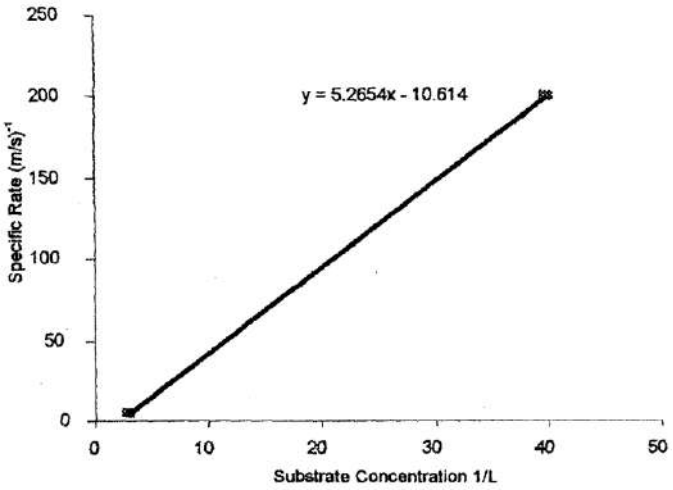

Fig. 9: Graph of Line-Weaver Burk for 10ml used lube oil

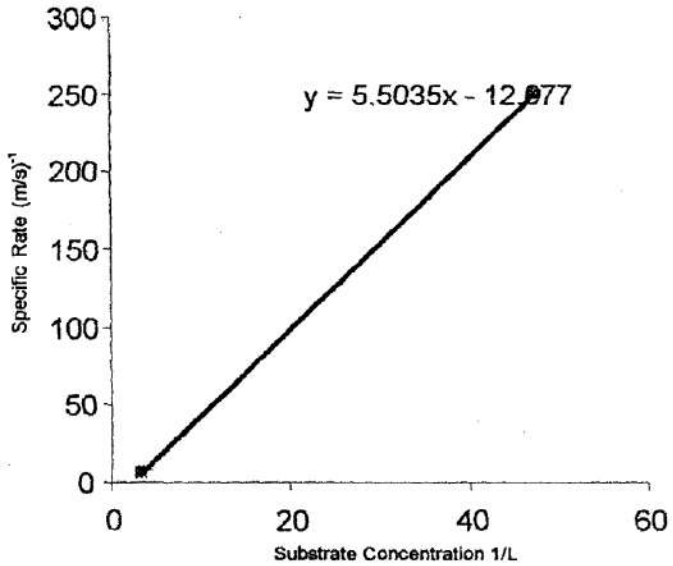

Fig. 10: Graph of Line-Weaver Burk for 20 ml of used oil

The graph obeys the Line-Weaver Burk plot. Therefore the maximum specific rate and the equilibrium constant (K_{L20}^{F}) can be evaluated mathematically as;

$$Y_{20} = 56.41x - 127.5 to\, give\, R_{max} = -1/127.5 = -0.008$$

$$K_L / R_{max} = 56.41, \qquad Therefore\, K_L = -0.78$$

For pollution with 30ml, Figure 4.8 illustrates the graph of $1/R_{30}^{F}$ against $1/L_{30}^{F}$ and it is seen that specific rate increases with decrease in reciprocal of substrate concentration. The graph obeys the Line-Weaver Burk Plot, whose equation is $Y_{30} = 47.38x - 150.18$

$$R_{max} = -1/150.18 = -0.007; K_L / R_{max} = 47.38; \; K_L = -0.78$$

Table 8: Theoretical calculation of a, b and t for different volumes of oil sampled

	Fresh oil			Used oil		
v	10 ml	20 ml	30 ml	10 ml	20 ml	30 ml
t	35 Days	60 Days	33.33 Days	400 Days	400 Days	55 Days
a	-2E-5	- 1E – 05	-6E -6	- 1E - 05	-2E -05	-2E-05
b	0.0007	0.0006	0.0002	0.004	0.008	0.0011

Where, a and b are numerical constants t stands for reaction time v is the reactor volume.

Table 9: Values for the theoretical determination of R_{max} and K_L

	Fresh oil			Used oil		
Vol	10 ml	20 ml	30ml	10 ml	20 ml	30 ml
Rmax	-0.015	-0.008	-0.007	-0.094	-0.083	-0.000009
KL	-0.78	-0.45	-0.32	-0.50	-0.46	-0.00036

R_{max} and K_L for used oil polluted soil

Figures 9 and 10 are typical Line-Weaver Burk Plots obtained for soils contaminated with used oils

i. For pollution with 10ml (Fig 9), the equation is;

$Y_{10} = 40.13 x – 112.65$ to give

$R_{max} = 1/112.65 = -0.094$; $K_L/R_{max} = 40.13$;KL= 0-0.50

ii. For population with 20ml (Fig. 10), the Line-Weaver Burk Plot equation is;

$Y_{20} = 5.5035x - 12.077$ to give

$R_{max} = -1/12.077 = -0.083$; $K_L/R_{max} = 5.5035$, $K_L = -0.46$

iii. For pollution with 30ml gave the Line-Weaver Burk Plot equation as;

$Y_{30} = 40.13x – 112.65.18$ to give

$R_{max} = 1/112.65 = 0.000009$; $K_L/R_{max} = 40.13K_L = -0.00036$

The various models developed in this research work could be applied to stimulate the biodegradation of fresh and used lube oil. The various results obtained from the theoretically developed models show that the results of the experiments were as expected as in the Line-Weaver Burk Plots.

Summary of the various values of R_{max} and K_L is shown in Table 9. It is observed generally that the values obtained for the maximum specific rate R_{max} for the degradation of phenol in fresh oil is lower than that of the used lube oil.

The R_{max} for phenol concentration obtained for soil polluted with 10ml, was observed to be lower and increased with increase in volume and the equilibrium constants K_L also followed the same trend. This goes to show that fresh lube oil degraded faster than used lube oil, which needs to be degraded to avoid environmental pollution (Ko et al., 2007; Luna et al., 2011; Quinchia et al., 2011; Wellman et al., 2001).

CONCLUSIONS

In this research, biokinetics of degradation of fresh and used lube oil have been examined. This includes determination of phenol concentration, microbial growth and physiochemical parameters for an aerobic degradation of fresh and used lube oil mixture in soil environment. Phenol concentrations in the used oil are higher than in the fresh oil before the experiment. This is apparently due to the oxidation which had already taken place during the use of the oil. Phenol has an inhibiting effect on the biodegradation of lube oils. Concentrations of phenol and hydrocarbon degraders build up rapidly to a maximum and then decreases. This shows that the initial attack of microbes in the lube oil, generates both phenol and hydrocarbon degraders. But due to the toxicity of phenol, the biodegradation of lube oil reduces resulting into the decline of both hydrocarbon degraders and the phenol yields.

The kinetic model formulated by Michaelis and Menten has been adapted in this work for the biodegradation reactions of fresh and used lube oil using changes in phenol concentration and hydrocarbon degraders as the key parameters for the models. The results obtained from the modeling, compared favorably with the experimental results. The models are particularly useful in predicting the time required for total extinction of the hydrocarbon degraders. Used lube oil has longer hydrocarbon degrader extinction time than fresh lube oil. The microbial growth rate model equations derived in this work could be applied for the;

i. Monitoring the rate of degradation of the fresh and used lube oil.

ii. Studying the mechanisms of biodegradation of fresh and used lube oiL

iii. Estimating the period of biodegradation for specific fresh and used lube oil present in petroleum industrial effluence or spills.

iv. Predicting the performance of fresh and used lube oil utilized.

v. Estimating the residence time for each lube oil component in the design of bio-treatment reactor.

vi. As a guide in monitoring bioremediation process

The derived model equations for the biodegradation of fresh and used lube oil could be applied in solving various environmental and other technical problems in the petroleum and chemical industries since in practice, industrial effluents are composed of mixtures of different lube oil.

NOMENCLATURE

C	Initial microbe concentration, (cfu/g)
d^{20}_4	Density of Lube oil, g/cc
K	Equilibrium constant
K_L	Equilibrium constant for Line-Weaver Burk Plot
M	Microbial population, (cfu/g)
R	Specific rate of substrates, $(ml/s)^{-1}$
R_{max}	Maximum specific rate, $(ml/s)^{-1}$
t	Reaction time, days
THC	Total hydrocarbons, (μg/g)
V	Volume (lube oil) of lube oil
(L)	Substrate (lube oil) concentration
$(L)_0$	Initial substrate concentration
Superscripts	
F	Fresh oil
U	Used oil
Greek Symbol	
η	Refractive index of lube oil

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