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# The Kinetics of Petroleum Hydrocarbon Degradation in Soil: The Concept of *Ponderosa* Lemon Integrity as Bio-Stimulant

Ukpaka Victor Chukwuemeka[1], Ukpaka Chukwuemeka Peter[2,*], Uzono Romokere Isotuk[3]

***Abstract***

*The kinetics of petroleum hydrocarbon degradation in soil: the concept of ponderosa lemon integrity as bio-stimulant with respect to the impact of preparation condition of room and sun-dried was monitored. The kinetic values of the effect of the biostimulant dosage were monitored and the data obtained from the experimental investigation was useful in the computation of the maximum specific rate of the substrate degradation and the dissociation constant of the substrate degradation. The results on TPH concentration showed that 50 g grape leaf room-dried (GLRD) had 64.24% and GLSD had 60.62% reduction compared to the control (15.61%). Increased dosage of 100 g GLRD had 97.08% and GLSD 66.21% which showed significant reduction across bio-stimulant at 42 days of treatment. The results on HUB for low dosage of 50 g GLRDB had $20.4\times10^4$ and GLSDB had $17.0\times10^3$. Increased dosage of 100 g GLRDB on HUB had $10.6\times10^3$ and GLSDB had $17.0\times10^3$. The results on HUB showed that GLRDB had better performance compared to GLSDB. Similar trend was observed with HUF on GLRDB compared to GLSDB. This study demonstrated the degradation potential of grape leaf on crude oil contaminated soil. The reciprocal of total petroleum hydrocarbon in comparison with GLRD and GLSD showed effectiveness in the remediation of crude oil impacted soil.*

**Keywords:** Kinetics, petroleum hydrocarbon, degradation, soil, concept, *Ponderosa* lemon

## INTRODUCTION

Crude oil as a material contains substances that are not environmentally friendly, and when in contact with the soil environment, induce the concentration of the useful nutrients that enhance plant growth [1]. The effect on agricultural plants depends on the degree of contaminants deposited in the soil. As the search for crude oil in Nigeria increases, there is tendency that the rate of crude oil spill will also increase which in turn decrease the available nutrient in the soil, by affecting the ecosystem [2]. The reason for treatment of contaminated soil environment is to ensure that the soil properties induced by spillage of crude oil is been restored by the application of bioremediation as recommended by various scientific authors and on the adaptation of four plants *Helianthus annuus, Paspalum conjugatum, Sorghum bicolor*, and *Tagetes erecta* in crude oil contaminated soil [3–6]. It was found out that two of the plants *Paspalum conjugatum* and *Sorghum bicolor* were effective in phytoremediation of crude oil contaminated soil. The application of moringa leaves' extract was found to be useful in enhancing crude oil polluted lands that facilitates the restoration of the contaminated soil [7]. Report on

***Author for Correspondence**
Ukpaka Chukwuemeka Peter
E-mail: peter.ukpaka@ust.edu.ng

[1]Research Student, College of Engineering, Computer Studies and Architecture, Department of Industrial Engineering, Lyceum of the Philippines University Cavite, Philippines
[2]Professor, Department of Chemical/Petrochemical Engineering, Rivers State University Port Harcourt, Rivers State, Nigeria
[3]Lecturer, Department of Chemical/Petrochemical Engineering, Akwa-Ibom State University Ikot-Akpaden, Akwa-Ibom State, Nigeria

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mango peel worked extremely well in crude oil contaminated soil thereby making it environment friendly and sustainable for plant growth. In view of the aforementioned studies, none has considered using grape leaf (*Ponderosa lemon*) in the treatment of crude oil contaminated soil [8–10].

The investigation of crude oil remediation in soil environment using the application of *Ponderosa lemon* (Grape) leaf to enhance the cleanup of contaminated soil is the purpose of this research. Indeed, the application of different agricultural materials has been used for the purpose of cleanup of contaminated soil by crude oil and other chemicals. Investigation conducted on crude oil remediation using *Mangifera indica* (mango seed) and their research revealed that mango seeds possess the characteristics of a biostimulant and the constituents that catalyzed the microbial actions, which induced and yielded high mitigation in terms of crude oil degradation in the different bioreactor setups [11–14].

This research was able to demonstrate the available nutrients present in the constituent of the biostimulant *Ponderosa lemon* (grape) in comparison to sun and room dried samples [15–17]. However, the investigation will show the percentage removal of the TPH and microbial activities upon utilization of the available nutrients supplied by the biostimulant (grape leaf) and this phenomenon will enhance contaminated soil restoration [18]. The findings from this research revealed that *Ponderosa lemon* (Grape) leaf can be grouped among the biostimulant recommended based on other researches carried out using different agriculturally based plants.

## MATERIALS AND METHODS

### Materials and Equipment

This research was achieved by using the following materials and equipment as stated, which includes: loamy soil, crude oil, biostimulator (*Ponderosa Lemon),* plastic containers (samples), Gas Chromatograph (GC) equipment, pH meter, reagents, funnels electronic balance, beaker, desiccators, flasks, sieves, mechanical machine, hand towel, oven, thermometer, electrical conductivity meter.

### Collection of Samples for Analysis

Samples were collected for analysis by using the Gas Chromatograph for determination of individual petroleum hydrocarbon concentration. The kinetic parameters were determined from the data that was obtained from this research work.

### Preparation of Sample and Set-up of Experiment

The bio stimulant was obtained from Erema Community in Egi clean in Ogba/Egbema/Ndoni Local Government Area of Rivers State, Nigeria and was transported to the Laboratory of Chemical/Petrochemical Engineering Department. The bio stimulant (*Ponderosa lemon*) grape leaf was washed and sun and room dried for the purpose of removal of impurities and reduction in the moisture content. After drying the bio stimulant, it was subjected with crushing to obtain the powder form of the remediant. The properties of soil and crude oil was analyzed and the experiment was set-up as shown in Figure 1.

### Particle Size Distribution (Hydrometer Method)

Air-dried soil (2 mm) of 51 g was poured into 250 ml beaker and 50 ml of calgon (sodium hexametaphosphate + sodium carbonate) was added to the soil sample. 100 ml of distilled water was added and stirred vigorously for 1 min using glass rod and allowed to stand for 30 min. The suspension was transferred into a mixer and mixed for 15 min at a medium speed. The suspension transferred into a sedimentation cylinder was made up to 1 l mark with distilled water measurements. The suspension was mixed in the cylinder by several vertical movements of the plunger. The cylinder was placed on a flat surface and time was recorded. The first reading was taken after 40 sec by sliding the hydrometer slowly into the suspension and the temperature reading was taken, the second reading was taken 2 h later and calculated to determine percentage of sand, silt and clay. Finally, the soil texture was also determined using soil textural triangle; according to United States Department of Agriculture (USDA), the soil textural classification scheme is shown in Figure 2.

 

**Figure 1.** Sun-dried and room-dried grape leaves.

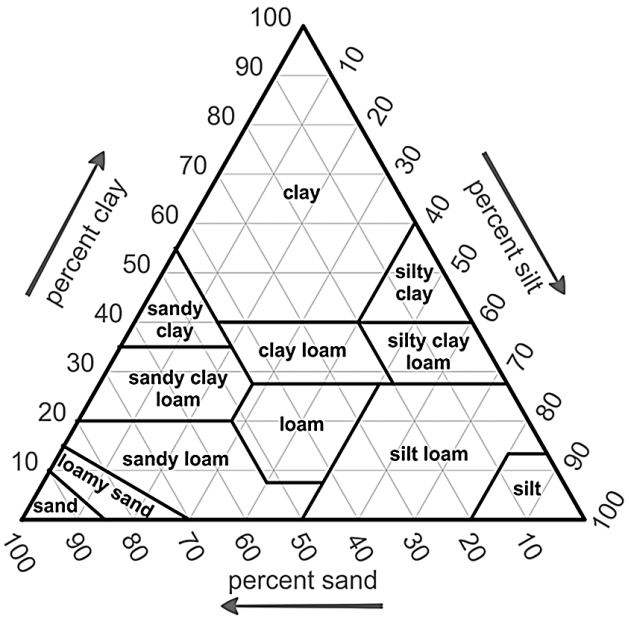


**Figure 2.** USDA soil textural classification.

 

**Determination of Percentage Reduction**

This was determined based on the initial and final concentration of the contaminant in the soil by using Eq. (1):

$$\text{Percentage Reduction (\%)} = \frac{\text{Ci} - \text{Cf}}{\text{Ci}} \times 100 \quad (1)$$

Where:

%= percentage reduction,

Ci= initial concentration of drill cuttings (mg/kg),

Cf= final concentration of the treated drill cuttings (mg/kg).

**Kinetic Model**

The rate of crude oil degradation and remediation can be monitored by developing the models that can predict the kinetic value of the process. The degradation of crude oil by the action of microorganisms and the products obtained are demonstrated below:

$$\text{Crude oil + ponderosa Lemon substrate + } O_2 \xrightarrow{\text{microorganism}} CO_2 + H_2O + \text{biomass + heat released} \quad (2)$$

The equation of the rate at which the crude oil degrades in terms of the first order with rate constant of K as well as total hydrocarbon content with respect to biostimulator is $THC_S$.

Therefore,

$$R = \frac{d[THC_S]}{dt} = -k[THC_s]^n \quad (3)$$

Where,

THC= concentration of total hydrocarbon content at time t,

k= the rate constant,

n= the order of the reaction,

$\frac{d[THC_S]}{dt}$ = the rate of change of total hydrocarbon over time.

Eq. (3) can be expressed as:

$$\frac{d(THC_S)}{(THC_S)^n} = -kdt \quad (4)$$

Integrating Eq. (4) for two cases n≠1 and n=1

***case 1: n≠1, integrating both sides, we have:***

$$\int \frac{d[THC_S]}{[THC_S]^n} = \int -kdt \quad (5)$$

From Eq. (5), the left-hand integral, we have:

$$\int \frac{d[THC_S]}{[THC_S]^n} = \frac{[THC_S]^{1-n}}{1-n} \quad (6)$$

From Eq. (6), we have:

$$\int -kdt = -kt + C \quad (7)$$

where, C is the constant of integration.

Integrating Eq. (6) and (7), we have:

$$\frac{[THC_S]^{1-n}}{1-n} = -kt + C \quad (8)$$

 

Simplifying Eq. (8), we have:

$$[THC_s]^{1-n}=(1–n)\ (–kt+C) \tag{9}$$

$$[THC_s]=[(1–n)\ (-kt\ +\ C)]^{\frac{1}{1-n}} \tag{10}$$

***Case 2: n=1, the simplifies to:***

$$\frac{d[THC_S]}{[THC_S]} = -kdt \tag{11}$$

From Eq. (11) integrating both sides, we have:

$$\int \frac{1}{[\mathrm{THC_S}]}\ \mathrm{d[THCs]}=\int -kdt \tag{12}$$

Thus, Eq. (12), gives:

$$\mathrm{In[THCs]}=–kt+C \tag{13}$$

Simplifying Eq. (13), we have:

$$[THCs]=e^{-kt+C}=Ae^{–kt} \tag{14}$$

Where $A=e^C$ is a constant.

From first-order, the concentration of hydrocarbon follows an exponential decay:

$$[THCs]=Ae^{–kt} \tag{15}$$

**Michaelis Menten and Monod's Model**

***Michaelis Menten Model***

The Michaelis Menten Model express the rate of degradation of the substrate (substrate kinetic) with respect to line and the kinetic model is given as:

$$R = \frac{R_{max}[TPH]}{K_{TPH}+[TPH]}$$

Or

$$R = \frac{R_{max}[S]}{K_S+[S]} \tag{16}$$

Where,

R is the specific rate of TPH degradation in (ppm/day),

$R_{max}$ is the maximum specific rate of TPH degradation $(ppm/day)^{-1}$,

$K_S$ is the grape leaf room-dried and sun-dried substrate or remediant.

**Mono's Model**

The concept of the Monod model is on the activities of the microbes responsible for the degradation of total petroleum hydrocarbon (TPH) with respect to time. The Monod's model expresses the activity of the microbes as stated below:

$$\mu = \frac{\mu_{max}[TPH]}{K_{TPH}+[TPH]} \tag{17}$$

Or

$$\mu = \frac{\mu_{max}[S]}{K_S+[S]} \tag{18}$$

Where,

$\mu$ is the specific rate of microbes $(cfu/g/day)^{-1}$,

$\mu_{max}$ is the maximum specific rate of microbes in (cfu/g),

$K_S$ is the equilibrium constant of microbes $(ppm)^{-1}$.

 

## RESULTS AND DISCUSSION

The results obtained from the research are demonstrated in Figures 3 and 4 as revealed below. Figure 3 showcases the trend of TPH concentration upon the effect of time with the application of 50 g dosages of the room-dried and sun-dried bio stimulants. The effectiveness of the bio stimulants was tested by comparing the performance of the bio stimulants with control system which does not receive any bio stimulant for the purpose of improving the mixture nutrient. Result showcases decrease in concentration of TPH with increase in time. Figure 3 further reveals high remediation in the bioreactor containing 50 g dosage of the room-dried bio stimulant than 50 g sun-dried dosage of bio stimulant.

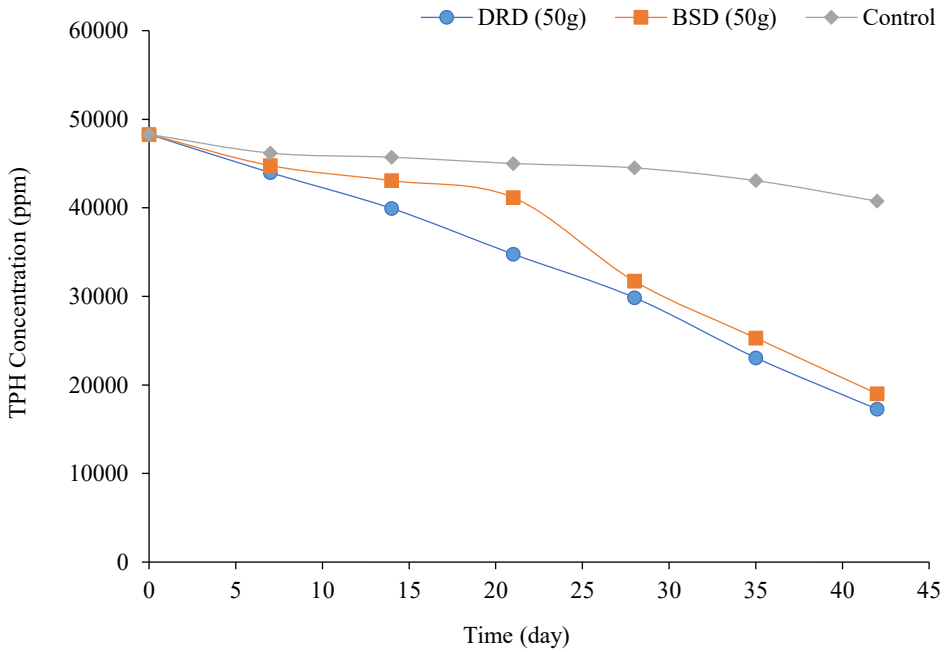


**Figure 3.** Comparison of TPH concentration against time for both sun and room dried application of the same dosage of 50 g of biostimulant and control.

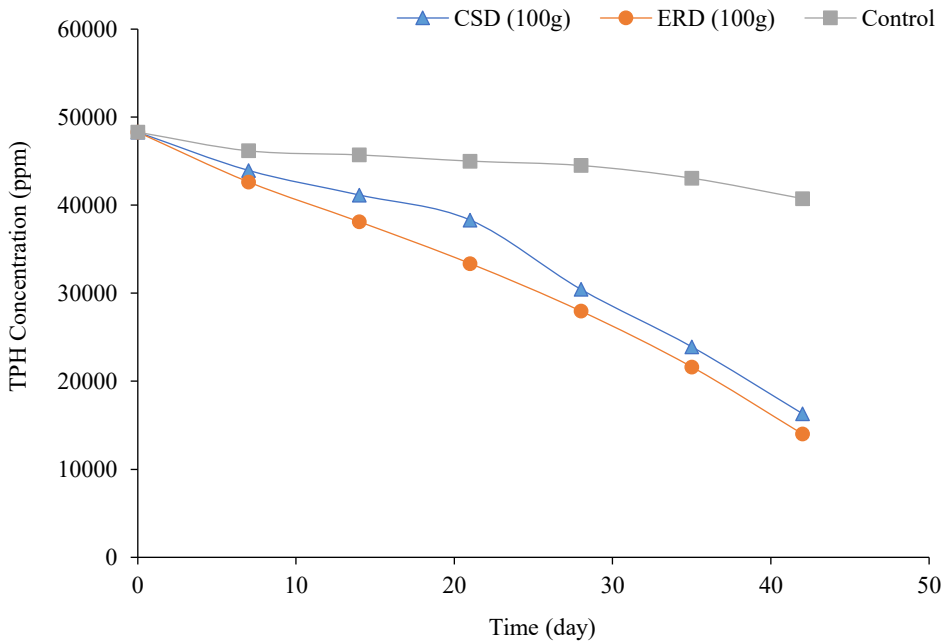


**Figure 4.** Comparison of TPH concentration against time for both sun and room dried application of the same dosage of 100 g of biostimulant and control.

 

Figure 4 showcases the trend of TPH concentration upon the effect of time with the application of 100 g dosage for room-dried and sun-dried bio stimulants. The effectiveness of the bio stimulants was tested by comparing the performance of the bio stimulants with control system which does not receive any bio stimulant for the purpose of improving the mixture nutrient. Result showcases decrease in concentration of TPH at contact time of 35 days amounting to 50.47% with increase in time due to increase dosages of GLSD that stimulated the microbes in the contaminated medium. The improvement of microorganism is a clear indication of how effective the substrate is to the contaminated soil.

**Results of Functional Parameters from Lineweaver Burk Plot Concept**

Figure 5 show the results presented from the functional parameters of 50 and 100 g GLSD and GLRD nutrients in the degradation of TPH. The developed Michaelis-Menten Model equation is expressed as: $r = \frac{r_{max}\,TPH}{K_{TPH}+TPH}$ and the Lineweaver Burk plot expression is given as: $\frac{1}{r} = \frac{K_{TPH}}{r_{max}\,TPH} + \frac{1}{r_{max}}$. The Lineweaver Burk plot line graphs express high degradation rate with 50 g nutrients (Figure 5). The reciprocal ($^1/_{TPH}$) of 50 g GLSD at 7, 14 and 21 days were $2.23367\times10^5$, $2.49574\times10^5$, and $2.7655\times10^5$. Meanwhile, there was increase in degradation rate due to contact time at 28 and 35 days $3.25546\times10^5$ and $3.80111\times10^5$ respectively, with most increase experienced at 42 days $4.75811\times10^5$. Report on *Mangifera indica* seed in bioremediation of contaminants using crude oil enhanced high degradation rate of hydrocarbon. Research highlighted increase in the removal efficiency of 70% on petroleum hydrocarbon from formulated composts of fresh wastes in acid sludge. The degradation rate equation may be a useful tool for rapid estimation of the degradation rate of TPH at any concentration in the bioremediation of TPH in crude oil-polluted loamy soil using 50 g GLSD. The differences may be caused by the population of enzymes interaction with the substrate. The results showed high coefficient of determination $R^2$=99.7% at contact time of 42 days.

Figure 6 displays the interaction of 100 g GLSD substrate to hydrocarbon contaminated soil. The application of Michaelis-Menten Model equation expressed as: $r = \frac{r_{max}\,TPH}{K_{TPH}+TPH}$ and the Lineweaver Burk plot expression given as: $\frac{1}{r} = \frac{K_{TPH}}{r_{max}\,TPH} + \frac{1}{r_{max}}$ showed high degradation rate with 100 g substrate as presented on a line graph (Figure 6). The reciprocal ($^1/_{TPH}$) of higher substrate 100 g GLSD at 7, 14 and 21 days were $2.27553\times10^5$, $2.42964\times10^5$, and $2.60994\times10^5$. Also, a more higher degradation rate occurred due to contact time at 28 and 35 days $3.28502\times10^5$ and $4.18112\times10^5$ respectively, with most increase experienced at 42 days $6.12763\times10^5$. It was established that the use of *Mangifera indica* seed in bioremediation of contaminated soil environment has demonstrated high performance for the restoration of contaminated soil. The degradation rate equation may be a useful tool for rapid estimation of the degradation rate of TPH at any concentration in the bioremediation of TPH in crude oil-polluted loamy soil using 100 g GLSD. Increased dosage of 100 g GLSD substrate on 100 ml crude oil contaminated soil had the coefficient of determination $R^2$=96.4% indicating effectiveness of the nutrient.

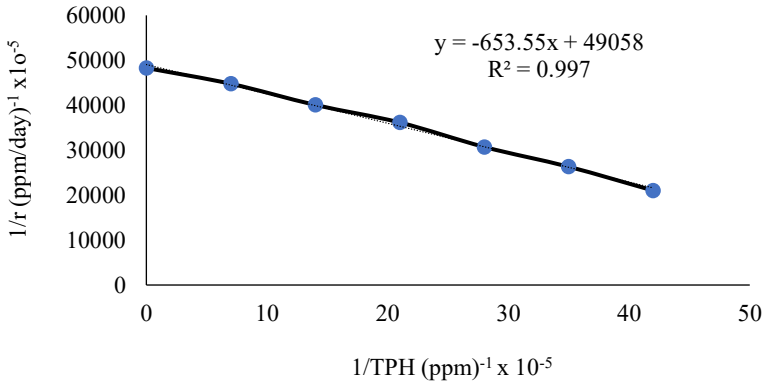


**Figure 5.** Computation of the recipient of TPH and specific rate of TPH degradation for GLSD (50 g) bioreactor setup for sun-dried bioremediant.

 

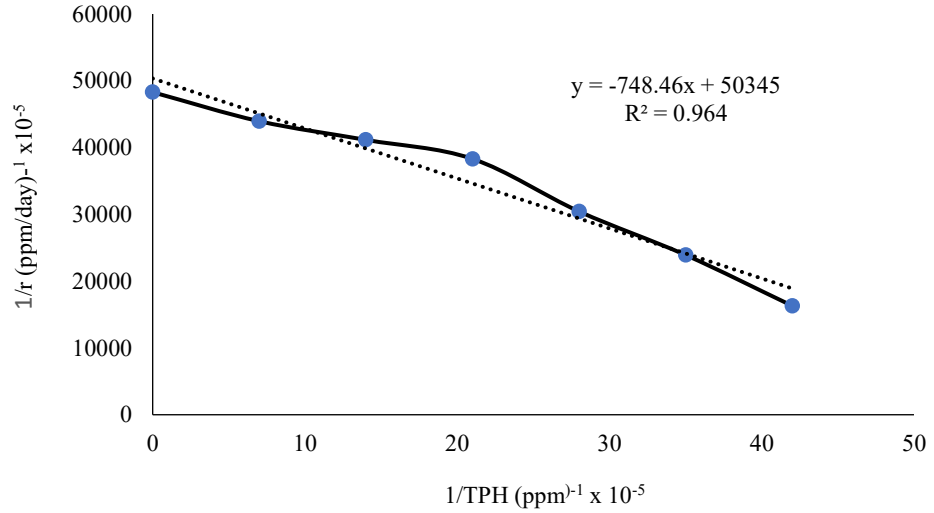


**Figure 6.** Computation of the recipient of TPH and specific rate of TPH Degradation for CSD (100 g) bioreactor set-up.

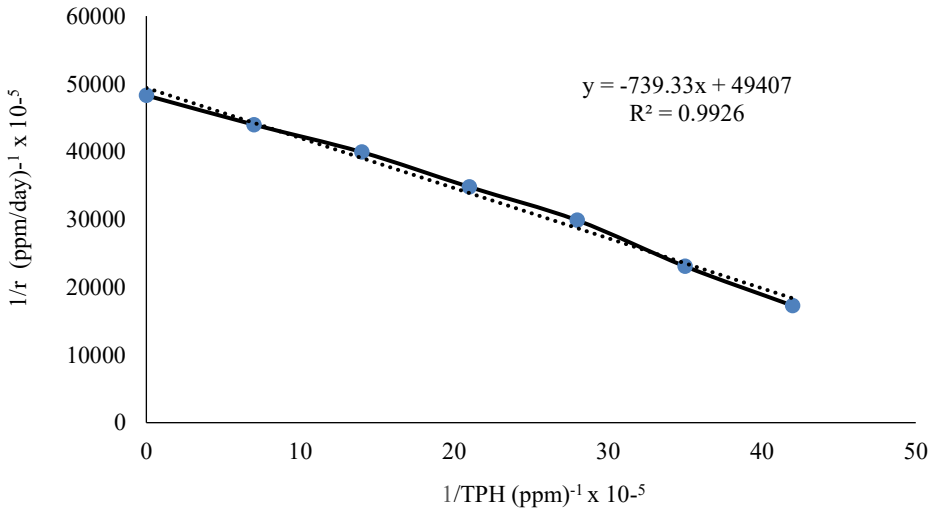


**Figure 7.** Computation of the recipient of TPH and specific Rate of TPH degradation for GLRD (50 g) bioreactor set-up.

Figure 7 shows the reciprocal ($^{1}/_{TPH}$) of minima dosage 50 g GLRD with contact time at 7, 14 and 21 days resulting to $2.27443\times10^5$, $2.50473\times10^5$, and $2.87557\times10^5$. Also, at 28 and 35 days $3.3489\times10^5$ and $4.33326\times10^5$ observed slightly higher degradation rate of TPH compared to 50 g GLSD substrate. The most increase was observed at 42 days $5.7907\times10^5$. Increase in the removal efficiency of 70% on petroleum hydrocarbon from formulated composts of fresh wastes in acid sludge. Figure 7 displays the interaction of 50 g GLRD substrate to hydrocarbon contaminated soil. The differences may be attributed to the amount of nutrient in the GLRD that stimulated the population of enzymes interacting with the TPH. The application of Michaelis-Menten Model equation expressed as: $r = \frac{r_{max}\,TPH}{K_{TPH}+TPH}$ and the Lineweaver Burk plot expression given as: $\frac{1}{r} = \frac{K_{TPH}}{r_{max}\,TPH} + \frac{1}{r_{max}}$ showed high degradation rate with 50 g substrate as presented on a line graph (Figure 7). The degradation rate equation may be a useful tool for rapid estimation of the degradation rate of TPH at any concentration in the bioremediation of TPH in crude oil-polluted loamy soil using 50 g GLRD. Similar trend was observed using 50 g dosage of GLRD substrate on 100 ml crude oil contaminated soil had the coefficient of determination $R^2$=99.26% indicating good performance of the substrate.

 

Figure 8 demonstrates the interaction of 100 g GLRD substrate to hydrocarbon contaminated soil. The application of Michaelis-Menten Model equation expressed as: $r = \frac{r_{max}\,TPH}{K_{TPH}+TPH}$ and the Lineweaver Burk plot expression given as: $\frac{1}{r} = \frac{K_{TPH}}{r_{max}\,TPH} + \frac{1}{r_{max}}$ showed high degradation rate with 100 g substrate as presented on a line graph (Figure 8). The reciprocal ($^{1}/_{TPH}$) of higher substrate 100 g GLRD at 7, 14 and 21 days were $2.08867\times10^{5}$, $2.16412\times10^{5}$, and $2.32509\times10^{5}$. Also, a more higher degradation rate occurred due to contact time at 28 and 35 days $2.38053\times10^{5}$ and $2.43595\times10^{5}$ respectively, with most increase experienced at 42 days $2.49681\times10^{5}$ (Figure 7). This report on *Mangifera indica* seed on crude oil enhanced degradation rate of hydrocarbon on soil environment revealed the high performance on environmental clean-up. Increase in the removal efficiency of 70% on petroleum hydrocarbon from formulated composts of fresh wastes in acid sludge was experienced. The degradation rate equation may be a useful tool for rapid estimation of the degradation rate of TPH at any concentration in the bioremediation of TPH in crude oil-polluted loamy soil using 100 g GLRD. Similarly, increase dosage of 100 g of GLRD substrate had the coefficient of determination $R^2$=99.16% indicating effective performance of the substrate on crude oil contaminated soil.

The trend on the inhibited interaction of microbes in the control. The application of Michaelis-Menten Model equation expressed as: $r = \frac{r_{max}\,TPH}{K_{TPH}+TPH}$ and the Lineweaver Burk plot expression given as: $\frac{1}{r} = \frac{K_{TPH}}{r_{max}\,TPH} + \frac{1}{r_{max}}$ showed slight degradation rate when compared with the GLRD and GLSD substrate as presented on a line graph (Figure 4). The reciprocal ($^{1}/_{TPH}$) results at 7, 14 and 21 days were $2.17\times10^{5}$, $2.22\times10^{5}$, and $2.19\times10^{5}$. Meanwhile, at 28 and 35 days $3.25\times10^{5}$ and $4.32\times10^{5}$ respectively, with most degradation at 42 days $7.45\times10^{5}$ (Figure 4). The reduction rate in the control option was based on existing microbes and some environmental factors like temperature and the duration of the study. The investigation on crude oil contaminated soil amended with mango leaf, poultry droppings and goat manure showed considerable TPH degradation efficiency of 76.6%. Similar work reported the use of *Mangifera indica* seed in bioremediation of contaminated soil environment has demonstrated high performance for the restoration of contaminated soil. And also, similar research highlighted increase in the removal efficiency of 70% on petroleum hydrocarbon from formulated composts of fresh wastes in acid sludge. The slow degradation rate was due to natural attenuation in the control media.

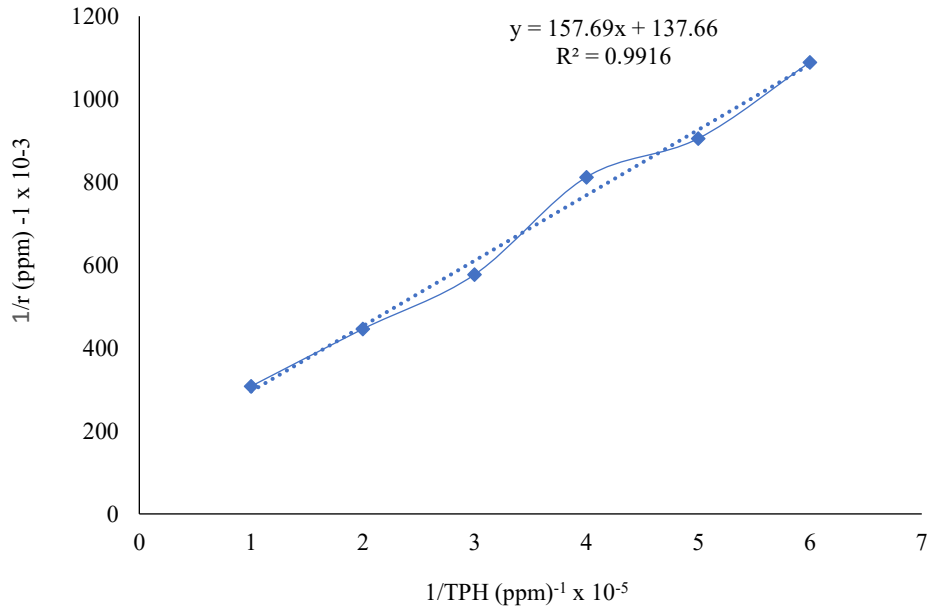


**Figure 8.** Computation of the Recipient of TPH and specific Rate of TPH Degradation for GLRD (100 g) bioreactor set-up.

 

## CONCLUSION

The following conclusions were projected from the findings on the investigation as illustrated below:

- Sun-dried and room-dried grape leaf nutrients influenced the degradation of TPH in the crude oil-contaminated loamy soil at 42 days of remediation. The 100 g of sun-dried did better than 50 g of the sun-dried, then, 100 g of room-dried degraded contaminant more than 50 g of room-dried, while 50 g of room-dried removed TPH more than 50 g of sun-dried, and, 100 g of room-dried did far better than 100 g sun-dried.
- The grape leaf (sun-dried and room-dried) enhanced the biodegradation of TPH in crude oil-contaminated soil. The nutrients degraded TPH up to 60.62, 66.21, 64.24, 97.08, and 15.61% for 50 g of GLSD, 100 g of GLSD, 50 g of GLRD, 100 g of GLRD and control samples, respectively, at the end of the 42nd day, it is obvious that 100 g of GLRD did better among treatments with 97.1% TPH reduction.
- The comparison of the percentage reduction of TPH by the application of biostimulant of 50 g dosage performance compared with the control sample for the remediation of 42 days revealed that the correlation value at 50 g of GLRD was higher than the 50 g of GLSD while 50 g of GLSD was higher than the control sample (50 g of GLRD>50 g of GLSD>Control. For 100 g dosage performance compared with the control sample, it shows that the correlation value at 100 g of GLSD was higher than the 100 g of GLRD while 100 g of GLRD was higher than the control sample (100 g of GLSD>100 g of GLRD>Control).
- For 50 and 100 g dosages performance compared with the control sample, it shows that the correlation value at 50 g of GLRD was higher than 100 g of GLSD, next was 50 g of GLSD, followed by 100 g of GLRD, which was higher than the control sample (50 g of GLRD>100 g of GLSD>50 g of GLSD>100 g of GLRD>Control).
- It is deduced from this study that as the time of remediation increased, the percentage reduction of the TPH also increased. It was observed that both 50 and 100 g Room-dried bio stimulant dosage degrade 96.41 and 99.37% while, 50 and 100 g dosage of Sun-dried bio stimulant removed 77.79 and 94.46% respectively. The percentage reduction for Room-dried bio stimulant is more effective in this study.

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