Journal of
Petroleum Engineering & Technology
https://journals.stmjournals.com/jopet
ISSN: 2231-1785 (Online)
ISSN: 2321-5178 (Print)
Volume 14, Issue 2, 2024
May–August
DOI (Journal): 10.37591/JoPET
Research JoPET

The Integrity of Eadic-Hofstee Plot Model to Predict the kinetic Parameters of Crude Oil Degradation using *Vernonia amygdalina* Stem

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Abstract

The integrity of Eadic-Hofstee concept was tested for the determination of the functional coefficients and parameters of crude oil degradation kinetics. The techniques enhanced the relationship between the substrate divided by the specific rate of the substrate degradation against substrate concentration (TPH). The investigation reveals the values of the biokinetic parameters of maximum specific rate of substrate degradation (V_{max}) and the equilibrium constant values of the substrate degradation (K_s). The concept of Eadic-Hofstee model was obtained from the reaction rate expression of Michaelis Menten model, which was further expressed in term of LineWeaver Burk Plot and rearrangement resulted in the Eadic-Hofstee model development. Biokinekic parameters determined are within the range of 0.0172 to 204.08 (ppm/day)$^{-1}$ for V_{max} and -1594.89 to -1750 (ppm)$^{-1}$ for K_s obtained from Michaelis Menten model and for Eadic-Hofstee the biokinetic parameters are within the range of 5.64525E-05 to 0.00055 (ppmm/day)$^{-1}$ for V_{max} as well as 0.0112865.53 to 0.35 (ppm)-1for K_s all parameters demonstrating the characteristics of the sun-dried performances. However, the room-dried revealed the following values of the biokinetic parameters as Vmax is 454.55 to 833.33 (ppm/day)-1 and Ks is -8447.27 to -20.75, which was obtained from Michaelis Menten model and Vmax is 9.32923E-05 to 0.046 (ppm/day)$^{-1}$ and Ks is 0.032707342 to 40.19 (ppm)$^{-1}$ for Eadic-Hofstee Plot model. The application of the model is found useful in evaluation of the biokinetic parameters and coefficients of crude oil degradation.

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Received Date: August 08, 2024
Accepted Date: August 29, 2024
Published Date: September 09, 2024

Citation: Abrahamr Peter Ukpaka, Victor Chukwuemeka Ukpaka, Joy Chukwuemeka Peter Ukpaka and Chukwuemeka Peter Ukpaka. The Integrity of Eadic-Hofstee Plot Model to Predict the kinetic Parameters of Crude Oil Degradation using *Vernonia amygdalina* Stem. Journal of Petroleum Engineering & Technology. 2024; 14(2): 37–47p.

Keywords: Integrity, Eadic-Hofstee Plot Model, predict biokinetic Parameters, crude oil, degradation, *Vernonia amygdalina* stem

INTRODUCTION

PAH-degrading microbes are pervasive in ecosystems where pollutants may serve as carbon sources, and seem to establish themselves soon after pollution occurs [1-2]. The reclamation of polluted land reduces the impact on groundwater by the pollutant as well as enhanced the soil quality [3-5]. Thus, natural degradation of pollutants in low-risk oil polluted sites are cost-effective and the rehabilitation, using biological procedures leads to another process [6] and they reported that substrate utilization pattern in the biological system changed upon addition of hydrocarbon. Previously pristine soil bacterial communities shifted to a predominantly *Pseudomonas sp* population with hydrocarbon degradation capability, thus demonstrating a natural bioremediation adaptation potential [7]. Similarly, research conducted in the same area demonstrate using a combination of biology™ and molecular methods and they found out that pollution removal by indigenous microbial communities at different soil levels was 48% in topsoil, 31% at 1m deep and 11% at 1.5m deep [8]. PAHs and phenols have been shown to be biodegradable under appropriate conditions [9]. However, the most readily degraded hydrocarbons are the n-alkanes with a relative molecular mass of up to n-C44 [10].

Bacteria are known to catabolize two- to four-ring PAHs as sole source of carbon, therefore, rendering them good candidates for site remediation applications [12-13]. This catabolism takes place using aromatic hydrocarbon deoxygenates within multicomponent enzyme systems [14]. The application of plant extract to catalyze the bioremediation process as well as the usefulness of the plant extracts in other area of engineering has been reported [15-17].

MATERIALS AND METHODS

Materials

The following materials listed below were used for the investigation; Containers: loamy soil (LS), crude oil (CO), digital weighing balance, remediant -*Vernonia amygdalina* stem, stirrer, polyethylene bag, hand trowel, conical flask, sampling bottles.

Sample Collection and Preparation

The loamy soil (LS) sample and *Vernonia amygdalina* stem collected from Elikpokwuodu community in Obio/Akpor Local Government Area of Rivers State Nigeria. The *Vernonia amygdalina* stem was subjected into room and sun-dry temperature and then samples were crushed into fine particles of $50\mu m$.

Experimental Concepts

Nine plastic containers labeled E_1, E_2, E_3, and E_4 containing mixture of 1kg of LS + 100ml of CO was prepared at first stage as well as another set of plastic containers labeled F_1, F_2, F_3, and F_4 to receive remediant of room and sun-dried as well as control sample labelled G without receiving the application of the remediant.

A_1: 1kg of LS + 100ml of CO + 25g of R of sun dried
A_2: 1kg of LS + 100ml of CO + 50g of R of sun dried
A_3: 1kg of LS + 100ml of CO + 75g of R of sun dried
A_4: 1kg of LS + 100ml CO + 100g of R of sun dried
B_1: 1kg of LS + 100ml of CO + 25g of R of room dried
B_2: 1kg of LS + 100ml of CO + 50g of R of room dried
B_3: 1kg of LS + 100ml of CO + 75g of R of room dried
B_4: 1kg of LS + 100ml of CO + 100g of R of room dried
C: 1kg of LS + 100ml of CO + no R added.

Where LS is denoted by loamy soil, CO is denoted by crude oil and R is denoted by remediant.

The following experimental concepts were carried out in this research as demonstrated below; 1kg of loamy soil (LS) samples were measured into each of the containers and then the initial concentration of the soil was determined and 50ml of crude oil (CO)was added into each of the container containing loamy soil (LS) and crude oil (CO) samples and then each bioreactor was stirred for complete mixing. The loamy soil (LS)samples inside each of the container was collected and analyzed after pollution.

The plant extracts (R- remediant) were measured in 25g, 50g, 75g and 100g for sun and room dried samples and introduced into each of the container and allowed for 7 days before samples were collected for TPH analysis using GC. The samples in each of the containers were analyzed in 7 days interval for period of 42 days to evaluate the performance of the plant extracts and also to properly examine the total petroleum hydrocarbon concentration.

The Eadic – Hofstee Model

The Eadic-Hofstee model was achieved by applying the Michael's Menten Model, which was further expressed to achieve the Lineweaver Burk-plot model or expression.

$V = \frac{V_{max}\,[S]}{K_S+[S]}$ Michael's Menten Model

$\frac{1}{V} = \frac{K_S}{V_{max}\,[S]} + \frac{1}{V_{max}}$ Lineweaver Burk-plot model

Therefore, expressing the Lineweaver burk-plot model in terms of V function and expression of this kind can be obtained, thus;

$$\frac{V_{max}}{V} = \frac{K_S}{[S]} + 1 \quad (1)$$

$$V_{max} = V\,\frac{K_S}{[S]} + V \quad (2)$$

Therefore,

$$V = V_{max} - K_S\,\frac{V}{[S]} \quad (3)$$

The Eadic-Hofstee plot of V against $V/[S]$ has an intercept of V_{max} and slope of - K_S.

RESULTS AND DISCUSSION

Experimental and Computation of Biokinetic Parameters of TPH

Table 1 and 2 demonstrates the Total Petroleum Hydrocarbon Concentration in Different Bioreactors of 25g, 50g, 75g, 100g and Control for the application of Sun-dried and room-dried remediation. The exercise was carried out in different time intervals of 0 (constant), 7, 14, 21, 28, 35 and 42 days respectively.

Table 3 (a, b) shows the kinetic parameters of the maximum specific rate of TPH remediation and the dissociation constant of the TPH in Michael Menten and Eadic-Hofstee model. However, the parameters of the functional coefficient vary as a result of variation in the TPH degradation or mitigation due to the action of the mass of *Vernonia amygdalina* added in each bioreactor as well as the microbial growth rate and other environmental factors.

Table 1. TPH Concentration in Different Containers for Application of Sun-Dried Remediation.

TPH Concentration (ppm)					
Time (Day)	***25g (Remediant)***	***50g (Remediant)***	***75g (Remediant)***	***100g Remediant)***	***Control***
0	35771.80	35771.80	35771.80	35771.80	35771.80
7	33052.46	32964.06	31816.61	31277.61	34491.20
14	30191.27	28950.22	2735.72	26183.55	32118.17
21	28461.12	23499.74	22951.10	21969.27	29893.82
28	20593.52	18122.07	17622.48	17022.83	26141.70
35	14638.19	1383.44	12314.26	11885.04	22550.15
42	8071.84	7200	6592.19	5004.72	17994.37

Table 2. TPH concentration in Different Bioreactors for Application of Room-Dried Remediant.

TPH Concentration (ppm)					
Time (Day)	*25g (Remediant)*	*50g (Remediant)*	*75g (Remediant)*	*100g (Remediant)*	*Control*
0	35771.80	35771.80	35771.80	35771.80	35771.80
7	32635.21	31851.72	31366.19	30475.27	34491.20
14	28162.77	26602.13	26149.28	25811.55	32118.17
21	23258.03	21011.66	20368.73	19174.24	29893.82
28	17114.51	15281.49	14072.17	13158.18	26141.70
35	10826.99	8857.20	8209.04	7138.43	22550.15
42	6933.47	1294.08	1162.96	1003.72	17994.37

Table 3. Biokinetic Parameters of the Michael's Menten, Hanes and Eadic-Hofstee Model value for Crude Oil Remediation with *Vernonia amygdalina* stem of both Sun and Room-Dried Remediant.

(a) Biokinetic Parameters of Sun Dried Remediant Effect					
S/N	**Dosage (Gram)**	**Michaelis Menten Model**		**Eadic-Hofstee Model**	
		$\mathbf{V_{max}}$ $\mathbf{(ppm/day)^{-1}}$	$\mathbf{K_S}$ $\mathbf{(ppm)^{-1}}$	$\mathbf{V_{max}}$ $\mathbf{(ppm/day)^{-1}}$	$\mathbf{K_S}$ $\mathbf{(ppm)^{-1}}$
1	25	204.08	-15940.89	6.4057E-05	0.013191339
2	50	526.32	-2826.68	5.64525E-05	0.011286553
3	75	0.0172	-456.66	0.001306067	0.948949779
4	100	625	-1750	0.00055	0.35
5	Control	60.61	-23470.30	6.765E-05	0.010983695
(b) Biokinetic Parameters of Room Dried Remediant Effect					
S/N	***Dosage (Gram)***	***Michaelis Menten Model***		***Eadic-Hofstee Model***	
		$V_{max}\,(ppm/day)^{-1}$	$K_S\,(ppm)^{-1}$	$V_{max}\,(ppm/day)^{-1}$	$K_S\,(ppm)^{-1}$
1	25	454.55	-8447.27	9.32923E-05	0.032707342
2	50	714.29	-2119.5	0.00538735	0.390168085
3	75	625	-4154.56	0.000238618	0.15218574
4	100	833.33	-20.75	0.046	40.19
5	Control	60.61	-23470.30	6.765E-05	0.010983695

Result of Biokinetic Model for Eadie-Hofstee Plot

From 1 to 9 shows the biokinetic model plot of Eadie-Hofstee. Their approach was also used in establishing and evaluating the maximum specific rate of TPH degradation and equilibrium constant of TPH.

Figure 1 shows the Eadie-Hofstee plot of V against $V/_S$ for dosage of 25g remediant. The regression equation obtained is given as $Y = 10719x + 350.59$ with $R^2 = 0.9389$ showing the percentage reliability value of 93.89% and the intercept value of 350.59 and slope of 10719 was achieved. However, the Eadic-Hofstee functional parameters and the coefficients were obtained as stated;

$V_{max} = 9.32923E - 05$ and $K_S = 0.032707342$

Therefore, in relationship to the Michael's Menten equation we have;

$$V = \frac{9.32923E - 05\ [S]}{0.032707342 + [S]}$$

Figure 2 shows increase in V_{50g} value with increase in $^{V}/_{[S]}$ for Eadic-Hofstee application for the determination of the functional parameters and coefficients. The regression model obtained revealed that $Y = 1856.2x + 724.23$ with $R^2 = 0.9948$ demonstrating the percentage reliability of 99.48%. From the regression equation intercept value is 724.23 and slope value = 1856.2. Indeed, the V_{max} = 0.00053875 (ppm/day)$^{-1}$ and $K_S = 0.390168085\ (ppm)^{-1}$, which reveals that from Eadic-Hofstee theorem, that the intercept is = V_{max} and slope = K_S

Therefore,

$V = V_{max} - K_S\ ^{V}/_{[S]}$ can be written as

$V = 0.00538735 - 0.390168085\ ^{V}/_{[S]}$ and in relationship to Michael Menten model, we have $V = \frac{V_{max}\,[S]}{K_S+[S]} = \frac{0.00538735\,[S]}{0.390168085+[S]}$

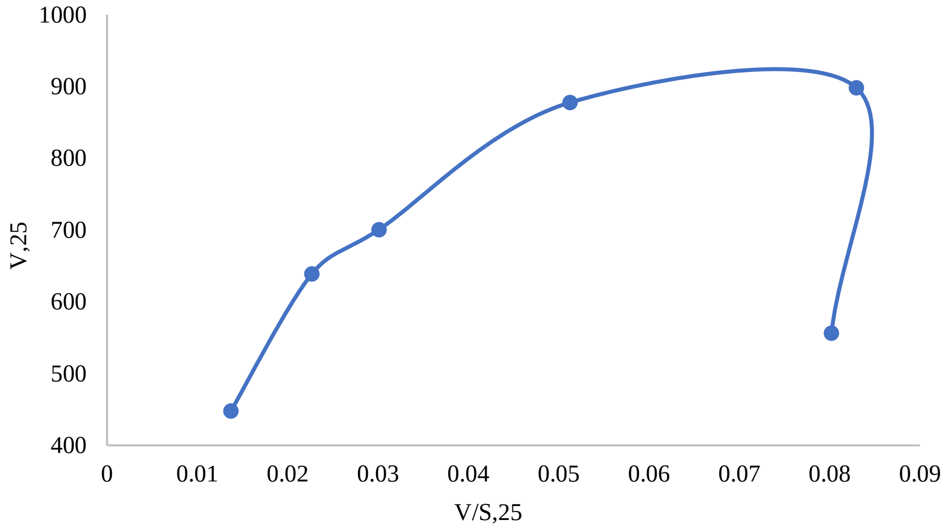

Figure 1. Eadic-Hofstee Plot of V_{25g} against $[^{V}/_{S}]_{25g}$ for 25g Dosage of *Vernonia amygdalina* of Room-Dried Remediant.

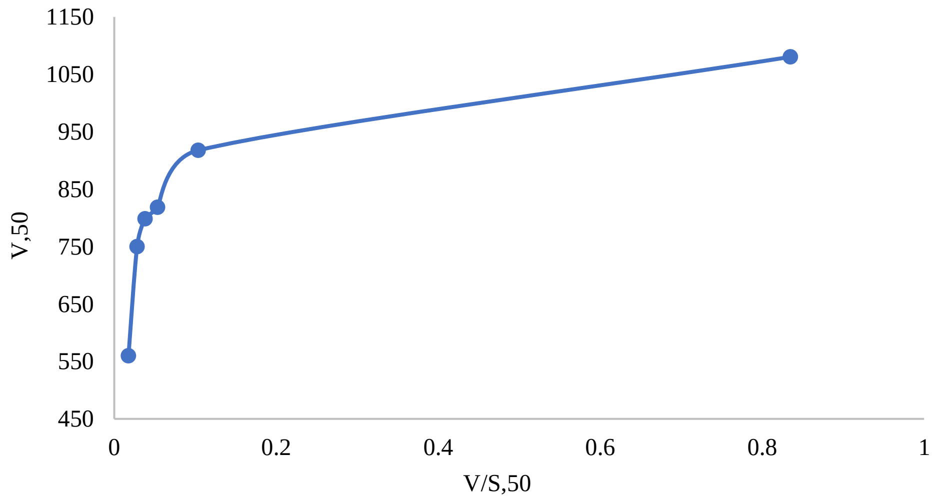

Figure 2. Eadic-Hofstee Plot of V_{50g} against $^{V}/_{[S]_{50g}}$ for 50g Dosage of *Vernonia amygdalina* of Room-Dried Remediant.

Figure 3 shows Eadic-Hofstee plot which revealed increase in the plot of V_{75} with increase in ${}^{V}/_{S_{75}}$ as experienced in terms of Lineweaver burk plot for the determination of V_{max} and K_S value. The regression equation of the line is given as $Y = 4190.8x + 637.78$ with $R^2 = 0.9573$ demonstrating the percentage reliability of 95.73%. However, from the regression equation obtained it is revealed that intercept value is 637.78 and slope value is 4190.8. The evaluated value of $V_{max} = 0.000238618\ (ppm/day)^{-1}$ and K_S = 0.15218574. The Eadic-Hofstee model revealed slope = K_S and intercept = V_{max}, therefore;

$V = V_{max} - K_S\ {}^{V}/_{[S]} = 0.000238618 - 0.15218574\frac{V}{[S]}$ and in relationship to Michael Menten model we have;

$$V = \frac{0.000238618\,[S]}{0.15218574 + [S]}$$

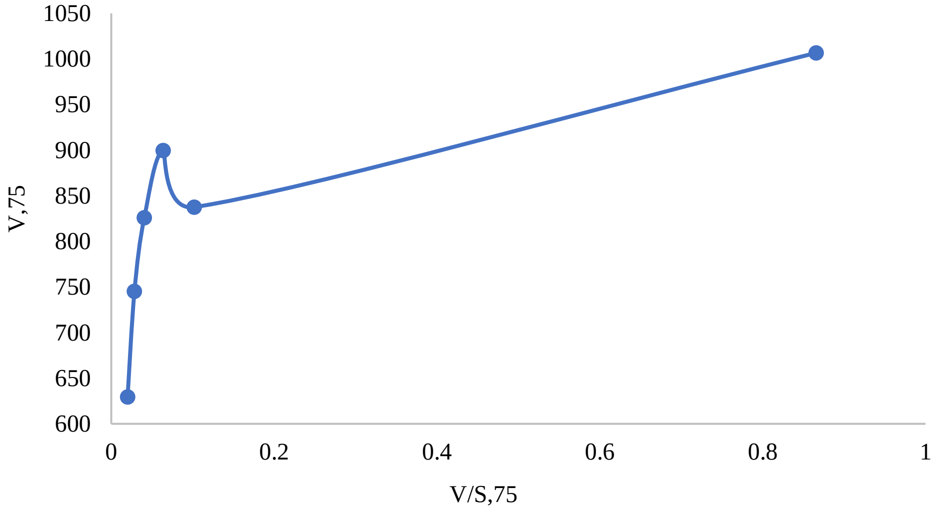

Figure 3. Eadic-Hofstee Plot of V_{75g} against ${}^{V}/_{[S]_{75g}}$ for 75g Dosage of *Vernonia amygdalina* of Room -Dried Remediant.

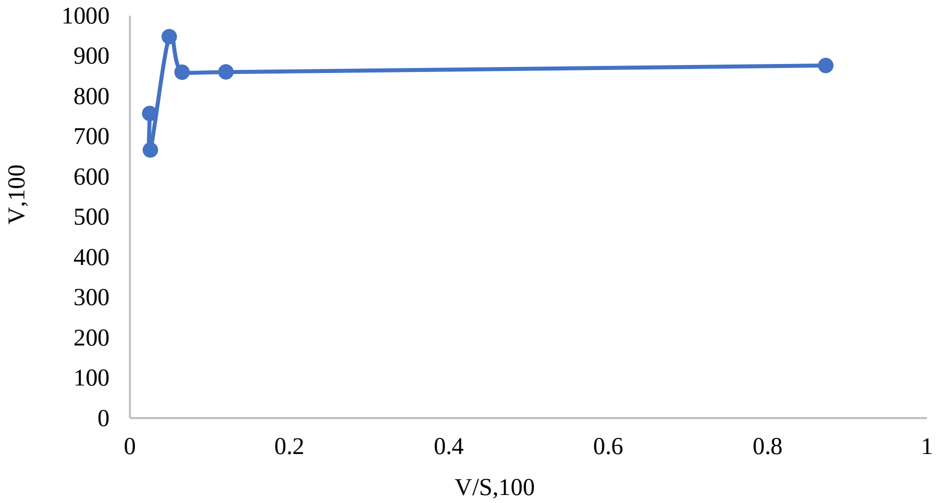

Figure 4. Eadic-Hofstee Plot of V_{100g} against ${}^{V}/_{[S]_{100g}}$ for 100g Dosage of *Vernonia amygdalina* of Room -Dried Remediant.

Figure 4 shows the Eadic-Hofstee plot relationship between V_{100g} and $V/[S]_{100g}$ of $100g$ dosage of *Vernonia amygdalina* of room dried remediant. Indeed, initial increase was experienced before sudden decrease and later increased in V_{100g} (specific rate) with increase in $V/[S]_{100g}$. The regression equation obtained is expressed as $Y = 21.342x + 857.73$ with $R^2 = 0.9989$ which shows the percentage reliability of 99.89%. The slope value is 21.342 and intercept value of 857.73 and the calculated value of $V_{max} = 0.046855965\ (ppm/day)^{-1}$ and $K_S = 40.189766666$. Recalling the Eadie-Hofstee equation of;

$$V = V_{max} - K_S \frac{V}{[S]}$$

where $V_{max} = 0.046855965\ (ppm/day)^{-1}$ and $K_S = 40.18976666\ (ppm)^{-1}$, Thus

$V = 0.046 - 40.19 \frac{V}{[S]}$ and Michael Menten equation becomes

$$V = \frac{0.046\ [S]}{40.19 + [S]}$$

Figure 5 shows an increase in V (specific rate) against V/S (specific rate / substrate). Degradation without the added of the remediant substrate in the bioreactor. Indeed, the regression equation in terms of relaionship between V and V/S is given as Y=14781x + 162.35 and R^2 = 0.9253 revealing the percentage reliability of 92.53%. The regression equation showcases the slope value of 14.781 and intercept value of 162.35 as well as the V_{max} value of 6.76544E-05 $(ppm/day)^{-1}$ and K_S = 0.010983695 $(ppm)^{-1}$. The Eadie-Hofstee equation is given as;

$$V = V_{max} - K_S\ V/[S]$$

Hence, the Eadic-Hofstee equation becomes $V = 6.765E - 05 - 0.010983695\ V/[S]$ and Michael Menten becomes

$$V = -\frac{6.765E - 05\ [S]}{0.010983695 + [S]}$$

Figure 6 showcases the Eadic-Hofstee plot of V_{25} (specific rate) and $V/[S]_{25g}$ (specific rate/substrate) degradation for 25g dosage of *Vernonia amygdalina* of sun dried remediants in a bioreactor. Indeed, the regression equation of the relationship is given as $Y = 15611x + 205.98$ with $R^2 = 0.9981$ revealing the percentage reliability value of 99.81%. From the quation of regression the slope value is 15611 and intercept value is 205.98 and the calculation of the $V_{max} = 6.40574E - 05(ppm/day)^{-1}$ and K_S = 0.013191339 $(ppm)^{-1}$. Substituting the value of V_{max} and K_S into the Eadic-Hofstee equation we have

$$V = V_{max} - K_S\ V/[S]$$

V = 6.40574E-05 – 0.013191339 $V/[S]$ and in terms of Michael Menten we have

$$V = \frac{6.40574E - 05\ [S]}{0.013191339 + [S]}$$

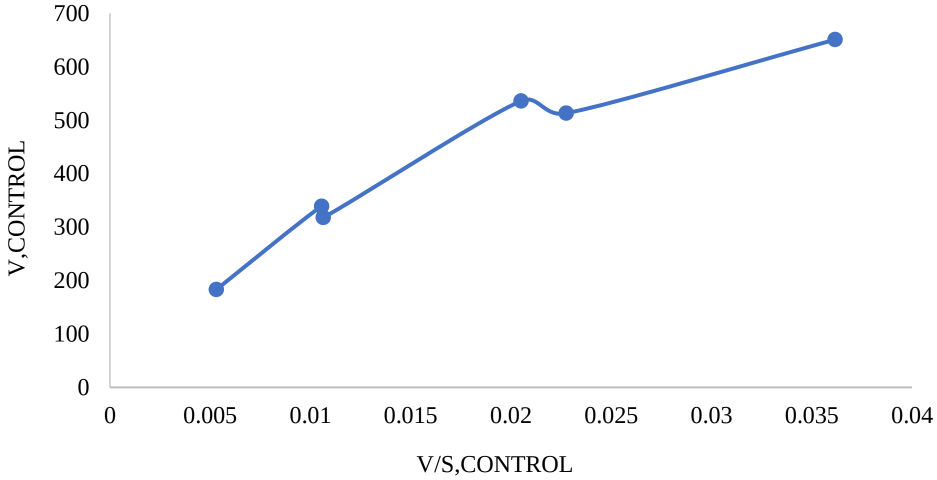

Figure 5. Eadic-Hofstee Plot of V_{50g} against $V/[S]_{control}$ of No Dosage of any Remediant in Crude Oil Degradation.

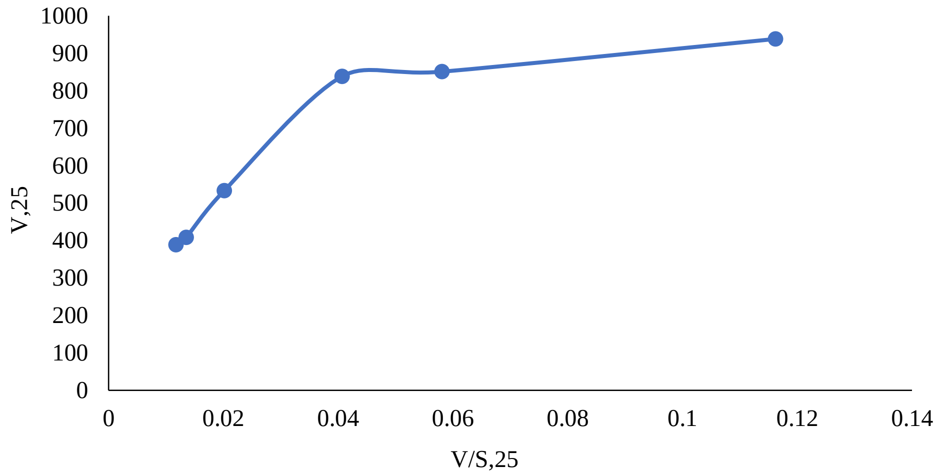

Figure 6. Eadic-Hofstee Plot of V_{25g} against $V/[S]_{25g}$ for 25g Dosage of *Vernonia amygdalina* of Sun-Dried Remediant.

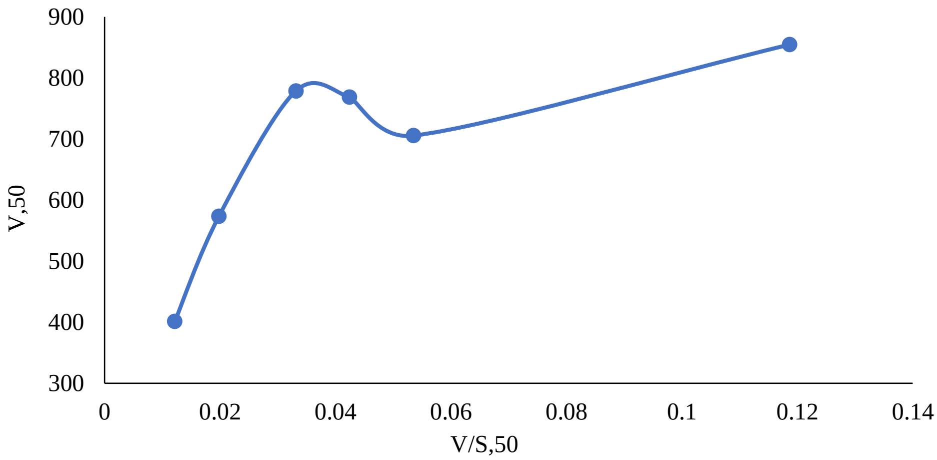

Figure 7. Eadic-Hofstee Plot of V_{50g} against $V/[S]_{50g}$ for 50g Dosage of *Vernonia amygdalina* of Sun-Dried Remediant.

Figure 7 shows the relationship between the plot of Eadic-Hofstee concept of V_{50g} against $^{V}/_{[S]_{50g}}$ of 50_g dosage of *Vernonia amygdalina* (remediant) of Sun-dried sample for crude oil degradation. However, the regression equation obtained revealed that $Y = 17714x + 199.93$ and $R^2 = 0.989$ as well as deterine the slope value of 17714 and intercept value is 199.93. The functional parameters were compulated and the following values achieved;

$V_{max} = 5.64525E - 05\ (ppm/day)^{-1}$ and $K_S = 0.011286553\ (ppm)^{-1}$. Substituting the parameters into Eadic-Hofstee equation we have;

$$V = V_{max} - K_S\ ^{V}/_{[S]}$$

$V = 5.6452E - 05 - 0.011286553\ ^{V}/_{[S]}$ and Michael Menten equation becomes

$$V = \frac{5.64525E{-}05\ [S]}{0.011286553 + [S]}$$

Figure 8 shows the Eadic-Hofstee plot of V_{75g} with $^{V}/_{[S]_{75g}}$ of 75_g dosage of *Vernonia amygdalina* (remediant) of sun-dried sample for crude oil remediation in a contaminated loamy soil. The regresion obtained from the plot is illustrated as $Y = 759.84x + 721$ with $R^2 = 0.9327$, which illustrates the percentage reliability of 93.27% and slope value of 759.84 and intercept value of 721.05. However, the $V_{max} = 0.001306067\ (ppm/day)^{-1}$ and $K_S = 0.948949779\ (ppm/day)^{-1}$ and $K_S = 0.948949779\ (ppm)^{-1}$. Substituting the value into the Eadic-Hofstee equation and Michael Menten equation we have the following equations;

$$V = V_{max} - K_S\ ^{V}/_{[S]} = 0.001306067 - 0.94894779\ ^{V}/_{[S]} \text{ and}$$

$$V = \frac{V_{max}\ [S]}{K_S + [S]} = \frac{0.001306067\ [S]}{0.948949779 + [S]}$$

Increase in V_{75g} (specific rate of substrate or TPH degradation) was experienced with increase in $^{V}/_{[S]}$ (specific rate of TPH degradation/substrate or TPH degradation).

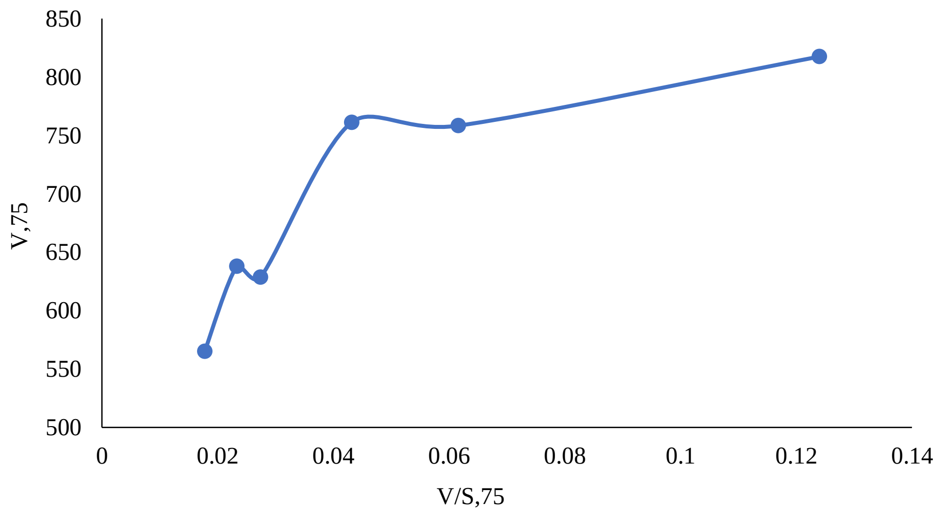

Figure 8. Eadic-Hofstee Plot of V_{75g} against $^{V}/_{[S]_{75g}}$ for 75g Dosage of *Vernonia amygdalina* (Remediant) of Sun-Dried for Crude Oil Degradation.

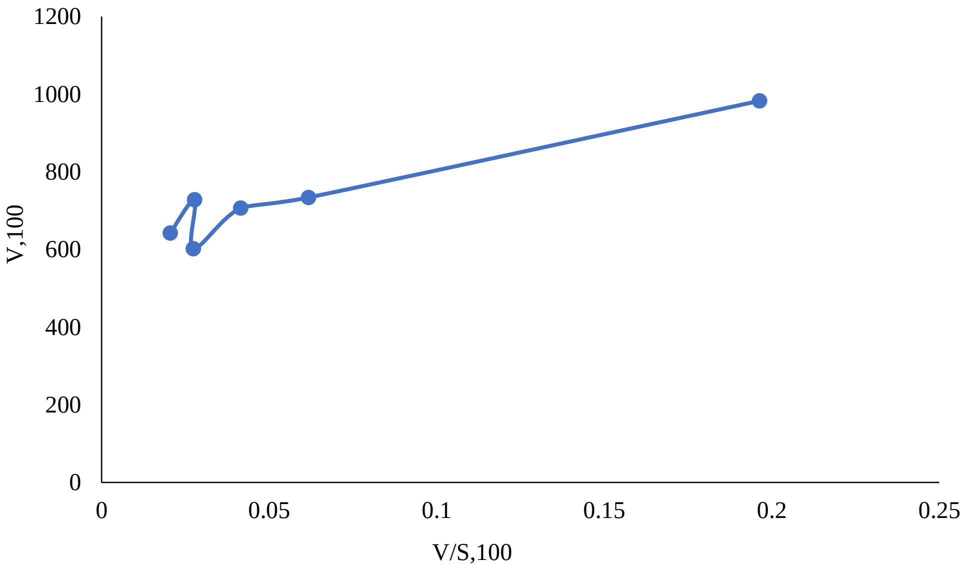

Figure 9. Eadic-Hofstee Plot of V_{100g} against $V/[S]_{100g}$ for 100g Dosage of *Vernonia amygdalina* (Remediant) of Sun -Dried for Crude Oil.

Figure 9 showcases the Eadic-Hofstee plot of V_{100g} in relationship to the plot of $V/[S]$ for 100_g dosage of *Vernonia amygdalina* (remediant) of sun dried for crude oil degradation in loamy soil environment. However, the regression equation achieved in the plot of V_{100g} versus $V/[S]$ is expressed as $Y = 1807.3x + 627.30$ and $R^2 = 0.9991$ revealing the percentage of reliability as 99.91% and slope value of 1807.3 and intercept value of 627.30. However, the calculated values of functional parameters are given as $V_{max} = 0.000553312\ (ppm/day)^{-1}$ and $K_S = 0.347097881\ (ppm)^{-1}$. Substituting these values into the Eadic-Hofstee equation as well as Michael Mentens, we have;

$V = V_{max} - K_S\ V/[S] = 0.00055 - 0.35\ \frac{V}{S}$ where slope = K_S and intercept = V_{max}

CONCLUSION

The integrity of Eadic-Hofstee model was tested for the evaluation of the biokinetic parameters of crude oil degradation in soil environment with the application of *Vernonia amygdalina* stem as remediant. The expression of the relationship of the plot of specific rate of TPH degradation or velocity rate of TPH and the reciprocal of the substrate (TPH) for the determination of the maximum specific rate of TPH degradation and equilibrium constant of TPH was determined as presented in this research. However, the following conclusion were showcased, such as:

1. The degradation rate of the TPH was faster in the room dried applied bio-stimulant than sun-dried
2. The biokinetic parameters of the Michaelis Menten and Eadic-Hofstee plot model demonstrates the various kinetic parameters of each bioreactor degradation process in terms of bioremediation occurrence in the system.
3. The process showcases know inhibition in each of the bioreactor as the results demonstrates TPH mitigation upon the action of the microbes.
4. Increase in microbial build-up was experienced
5. Eadic-Hofstee plot model is found useful in the determination of the biokinetic parameters as well as Michaelis Menten model

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