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Estimation of Degradation Rate Constant for Utilization in TPH Prediction during Soil Bioremediation

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Abstract

Crude oil pollution of soil is a major environmental concern that requires effective remediation. Bioremediation using microorganisms provides an eco-friendly approach. This study investigated the biodegradation of total petroleum hydrocarbons (TPH) in crude oil contaminated wet and dry soils treated with Pseudomonas sp. First order and Michaelis-Menten kinetics models were used to evaluate degradation rate constants. Results showed significant TPH reduction of up to 96.97% and 95.10% in wet and dry soils treated with Pseudomonas sp., respectively. The first order rate constant (k_d*) increased from 0.0073 day*$^{-1}$ *to 0.0394 day*$^{-1}$ *in wet soil and from 0.0057 day*$^{-1}$ *to 0.0298 day*$^{-1}$ *in dry soil with Pseudomonas sp. treatment. The maximum specific degradation rate and Michaelis-Menten constants also varied based on treatment. Comparison of experimental TPH values with model predictions showed the first order kinetics provided a better fit (*R^2 *0.9730-0.9869) than Michaelis-Menten model (*R^2 *0.8318-0.9590). By utilising the rate constants, the models can effectively predict TPH reduction over time during bioremediation.*

Keywords: Bioremediation, TPH, Kinetics models, Degradation rate constant

1. Introduction

Contamination of soil with crude oil is a major environmental problem requiring effective remediation approaches (Agarry *et al*., 2013). Total petroleum hydrocarbons (TPH) are the major toxic components in crude oil that persist in the soil (Varjani *et al*., 2015). Bioremediation using microorganisms provides an eco-friendly solution by transforming TPH into less toxic compounds (Borah and Yadav, 2016). Understanding the kinetics of TPH degradation is essential for evaluating and predicting the efficiency of bioremediation (Asgari *et al*., 2017).

Kinetic models such as first order and Michaelis-Menten equations have been widely used to describe TPH degradation in soil environment during bioremediation (Asgari *et al*., 2017; Amagbo and Ere, 2019). With use of these models, the rate constants areevaluated and used to determine the speed of substrate utilization and residual pollutant concentration over time (Obinna *et al*., 2015; Asgari *et al*., 2017). Comparison of experimental TPH data with model predictions validates the appropriateness of the kinetic model (Aghalibe *et al*., 2017). Estimation of accurate rate constants is vital for reliable prediction of TPH degradation and determining treatment duration (Amagbo and Ere, 2019).

This study investigated TPH degradation in crude oil contaminated wet and dry soils treated with varying levels of Pseudomonas sp. The first order and Michaelis-Menten models were used to evaluate degradation rate constants. Model predicted TPH values were compared with experimental results to determine the suitability of the kinetic models. The estimated rate constants can be further utilized for predicting TPH reduction during bioremediation of crude oil polluted soils.

2. Materials and Methods

2.1 Soil contamination and treatment

Wet and dry soil samples were artificially contaminated with crude oil to achieve initial TPH concentrations of 35981.09 mg/kg and 32563.39 mg/kg, respectively. The contaminated soils were treated with 0 ml (control) and 50 ml of *Pseudomonas sp*. and incubated at room temperature for a period of 84 days.

2.2 TPH analysis

Representative samples were collected at 14 days interval to analyse the TPH concentrations in the treated and control soils. Samples were analysed using gas chromatography according to ASTM D7066-04 method (ASTM, 2015).

2.3 Kinetic models

The first order rate kinetics and the Michaelis-Menten equation were used to evaluate the TPH degradation rate based on experimental data. This was performed by determining the constant(s) in both equations.

The first order rate kinetics is expressed as:

$$\ln C_{TPH(t)} = \ln C_{TPH(o)} - k_d t \tag{1}$$

Where, $C_{TPH(o)}$ and $C_{TPH(t)}$ are initial and final TPH concentrations (mg/kg), t is time (days) and k_d is the first order rate constant (day^{-1}). A plot of $\ln C_{TPH(t)}$ against$t$, gives a straight line graph with slope equivalent to "k_d" and intercept equivalent to $\ln C_{TPH(o)}$.

The Michaelis-Menten equation is expressed as:

$$r_{TPH} = \frac{\mu_{\max} S}{K_s + S} \tag{2}$$

Upon linearization, equation (2) becomes:

$$-\frac{1}{r_{TPH}} = -\frac{dS}{dt} = \frac{1}{\mu_{\max}} + \frac{K_s}{\mu_{\max}}\left(\frac{1}{S}\right) \tag{3}$$

Where, $\mu_{\max}$ is the maximum specific degradation rate (mg/kg.day), S is the concentration of TPH (mg/kg), t is time (day) and K_s is the degradation rate constant relating to Michaelis-Menten (mg/kg).Lineweaver-Burk plot was used to determine $\mu_{\max}$ and K_s. That is, a plot of $\frac{1}{r_{TPH}}$ against $\frac{1}{S}$ will give the slope as $\frac{K_s}{\mu_{\max}}$ and the intercept as $\frac{1}{\mu_{\max}}$.

The determined constants were plugged into the respective equation for both wet and dry soils, which were then utilised to predict the TPH level.

3. Results and Discussion

The predicted TPH from both models were compared with experimental results obtained for both wet and dry soils under natural attenuation and *Pseudomonas sp*. treatment.

3.1 TPH degradation

The TPH concentrations recorded over the period of investigation in *Pseudomonas sp*. treated and untreated crude oil polluted wet and dry soils are shown in Table 1.

Table 1: TPH degradation in wet and dry soils

Time (Day)	**Control**		**50ml Pseudomonas *sp.***	
	Wet soil TPH (mg/kg)	Dry soil TPH (mg/kg)	Wet soil TPH (mg/kg)	Dry soil TPH (mg/kg)
0	35981.09	32563.39	35981.09	32563.39
14	29969.94	27503.04	14969.93	14031.56
28	29553.10	24930.53	11699.25	12579.24
42	28650.77	23741.97	9084.14	10973.86
56	25820.46	21234.59	4462.37	7499.35
70	23153.36	18847.69	2051.28	4864.97
84	21093.62	16965.53	1089.15	1595.61

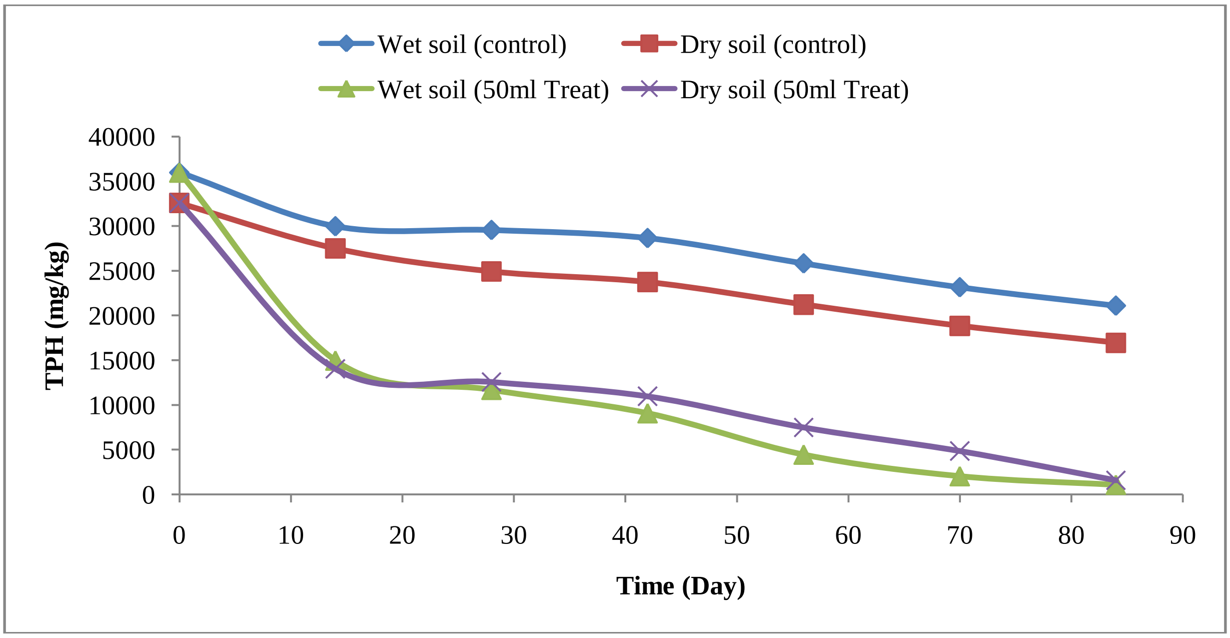

Figure 1: Variation in Specific TPH Degradation in Wet Soil with Time

Figure 1 shows the profiles of TPH degradation in *Pseudomonas sp*. treated and untreated wetand dry soil samples. The profiles indicated that TPH removal increased over time in both treated and control soils. While there was a decrease of TPH in soils without treatment (control samples), the level of degradation is comparatively low. Whereas, the degradation rate of TPH concentration in the samples treated with *Pseudomonas sp*. was high in both soils. Thus, the *Pseudomonas sp*. treatment sample showed significantly higher degradation up to 96.97% and 95.10% in wet and dry soils, respectively compared to 47.90% and 41.38% in untreated soils.

The low level of TPH degradation recorded in the untreated soils is expected because of limited nutrients required for catabolic activities to take place in the soils. According studies, the population of hydrocarbon utilising bacteria in soil influenced the degradation rate of hydrocarbon substrates (Hamoudi-Belarbi *et al*., 2018; Ere and Amagbo, 2019). Therefore, the enhanced degradation of TPH in *Pseudomonas sp*. treated wet and dry soils can be attributed to multiplication of the hydrocarbon utilization microbial community. However, the results obtained were consistent with earlier study on the degradation of TPH in soil environment amended microorganisms or microorganism stimulating agents (Hamoudi-Belarbi *et al*., 2018; Yu*et al*., 2020; Ere *et al*., 2020).

Generally, the TPH degradation in the control samples is low compared to the soil treated with *Pseudomonas sp*., implying that under natural attenuation, the TPH removal from crude oil polluted soil may take a very long time before the soil can be restored to its natural state.

3.2 Evaluation of first order degradation rate constant

The TPH degradation rate constant for first order rate kinetics was determined by comparing equation (1) with regression equations inFigures 2and 3.

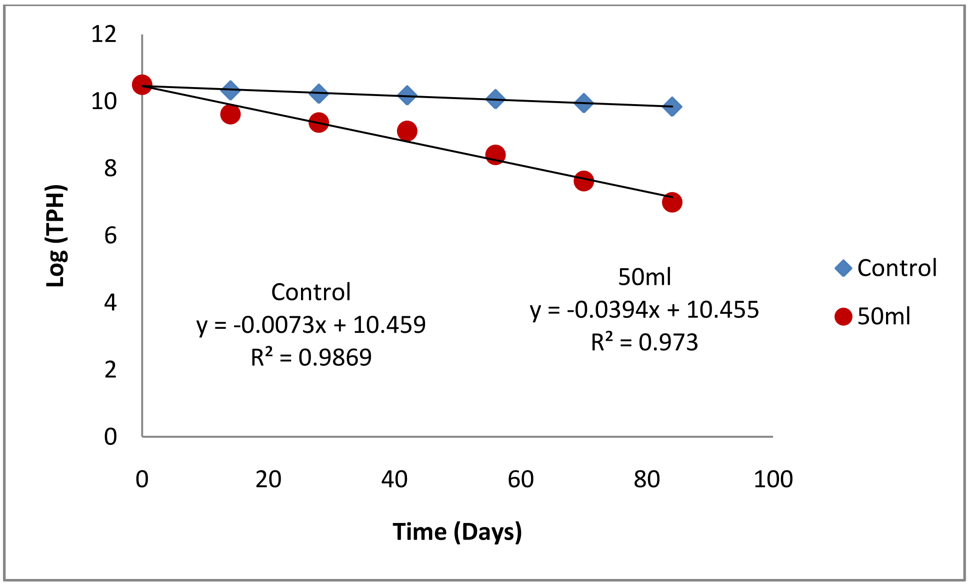

Figure 2: First order rate data evaluation for wet soil

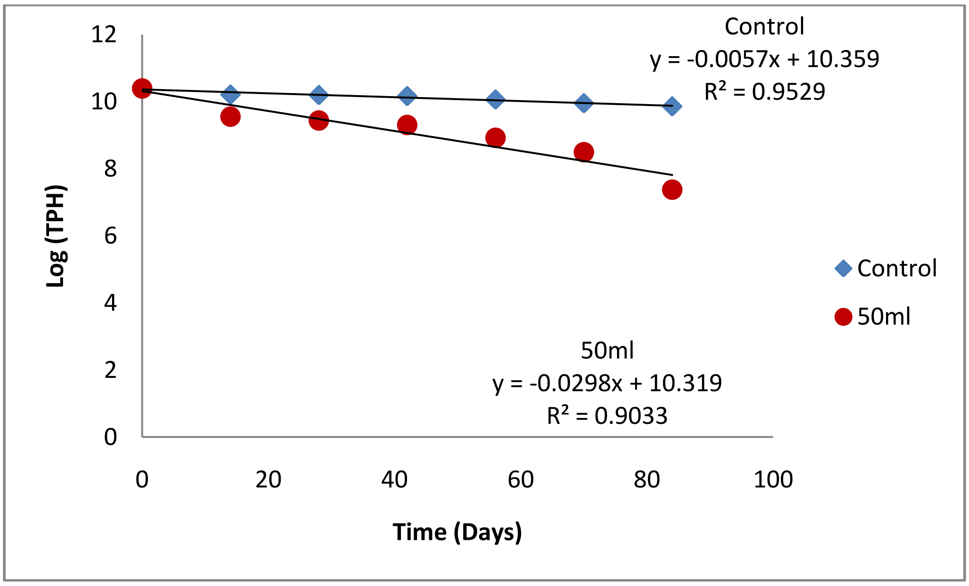

Figure 3: First order rate data evaluation plot for dry soil

The calculated degradation rate constant from Figures 2 and 3 were substituted into the first order rate kinetic model in equation (1) to obtain the predictive model for control (natural attenuation) and 50 ml *Pseudomonas sp.* treatment samples in wet and dry soils as summarized in Table 2.

Table 2: First Order Degradation Rate Model

***Pseudomonas sp.* (ml)**	**Wet Soil**	**Dry Soil**
0	$C_{TPH} = 35981.08e^{-0.0073t}$	$C_{TPH} = 32563.39e^{-0.0057t}$
50	$C_{TPH} = 35981.08e^{-0.0394t}$	$C_{TPH} = 32563.39e^{-0.0298t}$

From Table 2, it was observed that the degradation rate constant, *k* increased with increase in quantity of *Pseudomonas sp.* added in the soil samples. In Figures 2 and 3, the variable *x* in the regression equation stands for time, while the coefficient of *x* is the TPH degradation constant k_d. The negative sign in the equation indicates that TPH decreased with time, and $R^2$is the correlation coefficient, which shows the degree of correlation between the experimental and the predicted values. Moreover, the TPH degradation rate constant obtained are 0.0073day^{-1} and 0.0057 day^{-1}for wet and dry soils control sample, while in the samples treated with 50 ml of *Pseudomonassp.*,TPH degradation rate constant were obtained as 0.0394 day^{-1} for wet soil and 0.0298 day^{-1} for dry soil. This implied that the soil samples treated with *Pseudomonas sp*. increased the k_d value, which indicates a faster degradation rate. Similar range of rate constants have been reported for soils treated with nutrients and microbial inoculants (Aghalibe *et al*., 2017; Amagbo and Ere, 2019).

3.3 Evaluation of Michaelis-Menten model constants

Similarly, the maximum specific rate constant and the constant relating to Michaelis-Mentenin equation (3) were determined using the Linewaver-Burke plot shown in Figures 4 to 7. Thus, from the regression equations the constants were evaluated at the different treatment samples with and without *Pseudomonas sp*.

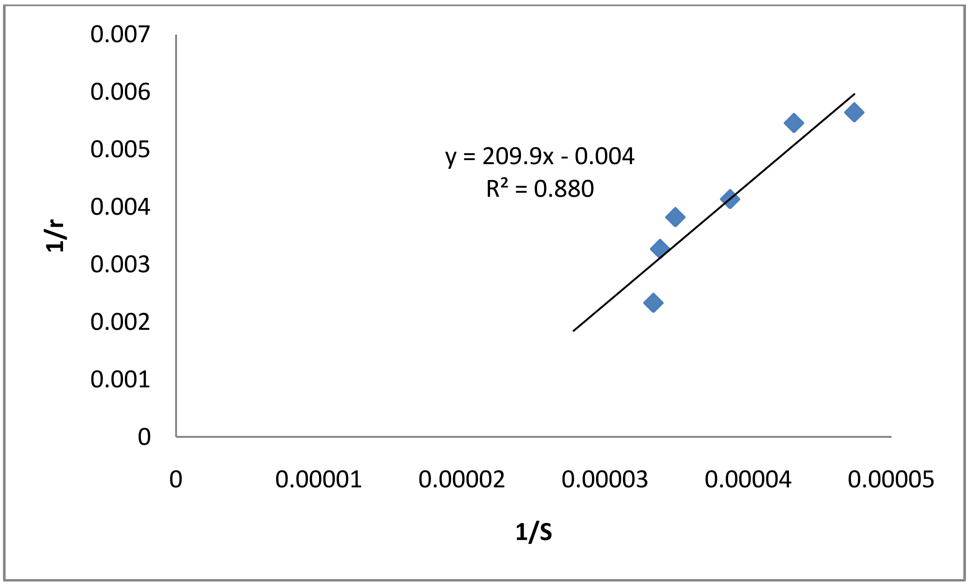

Figure 4: Linewaver-Burke Plot for Wet Soil Control Sample

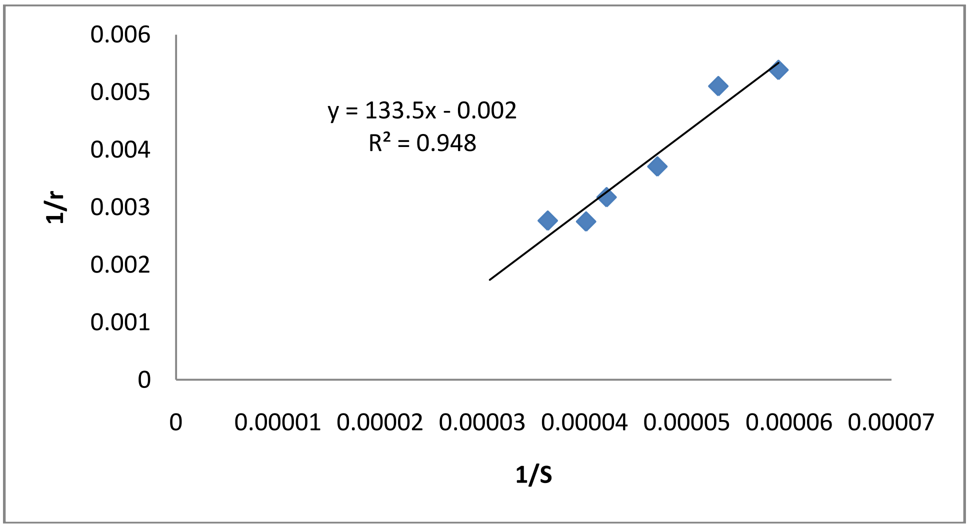

Figure 5: Linewaver-Burke Plot for Dry Soil Control Sample

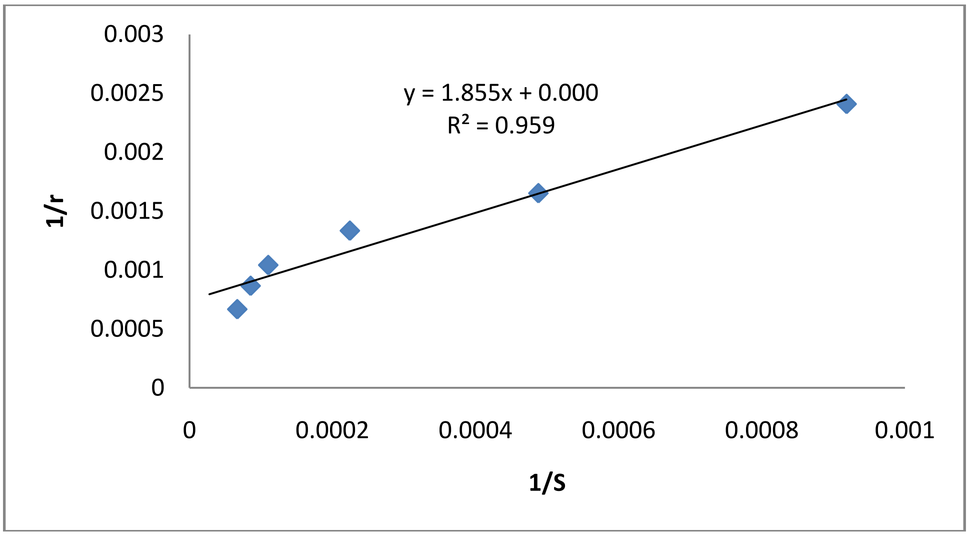

Figure 6: Linewaver-Burke Plot for Wet Soil at 50ml Treatment

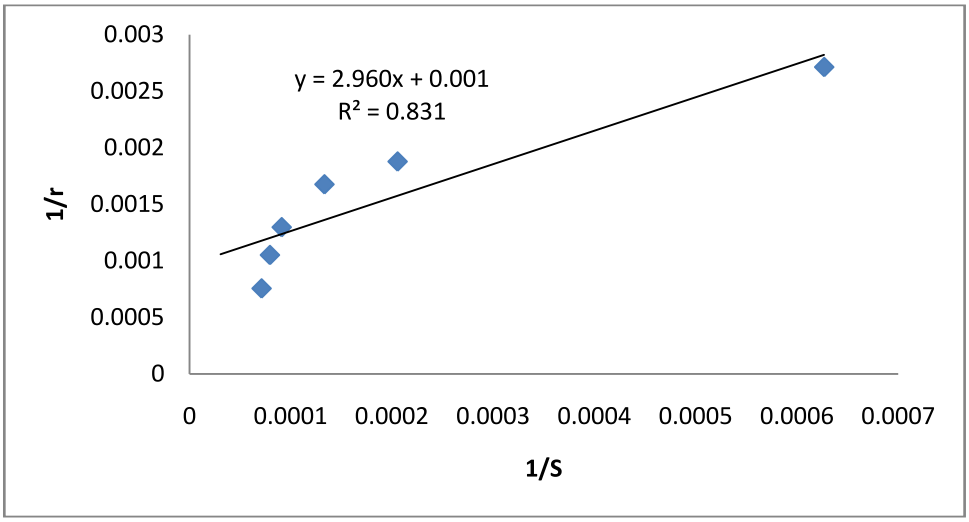

Figure 7: Linewaver-Burke Plot for Dry Soil at 50ml Treatment

Figures 4 to 7 show the plots for estimation of the maximum TPH degradation specific rate constant, U_m and the constant K_s for the Michaelis-Menten rate kinetic model. Thus, from the regression equations on the plots, the constants were evaluated for the different treatment options. Upon substitution of the evaluated Michaelis-Menten constants into equation (2), the Michaelis-Menten model corresponding to the treatment option were obtainedas shown in Table 3.

Table 3: Michaelis-Menten Model with the Evaluated Constants

Quantity (ml)	**Wet Soil**	**Dry Soil**
Control	$r_{TPH} = \frac{-250C_{TPH}}{-52480 + C_{TPH}}$	$r_{TPH} = \frac{-416.67C_{TPH}}{-55629 + C_{TPH}}$
50	$r_{TPH} = \frac{1428.57C_{TPH}}{2651 + C_{TPH}}$	$r_{TPH} = \frac{1000C_{TPH}}{2960.30 + C_{TPH}}$

From Table 3, it can be observed that the values of maximum specific rate constant ($\mu_{\max}$) and the Michaelis-Menten constant (K_s) for the treated sampleswere positive, unlike the control samples. This indicate the microbial influence on the substrate (crude oil contaminant), leading to accelerated rate of TPH degradation, while the negative $\mu_{\max}$ and K_s values obtained for the control soils indicate minimal degradation. The variable *x* in the regression equation represents the inverse of TPH($\frac{1}{S}$), while the coefficient of *x* represents $\frac{K_s}{\mu_{\max}}$. The constant of the regression equation represents the inverse of maximum specific rate, $\frac{1}{\mu_{\max}}$, while the $R^2$is the correlation coefficient, which shows the degree of correlation between the experimental and the predicted values.

The application of Michaelis-Menten equation for prediction of TPH degradation rate in crude oil contaminated soil amended by *Pseudomonassp*. was reported by Obinna *etal*. (2015), but the evaluated values of maximum specific rate constant, $\mu_{\max}$ (0.236±0.018) and the Michaelis-Menten constant, K_s (1.370±0.368) reported were below the values obtained in this study. However, variable kinetic constants with high degree of fit with experimental values have been reported by other studies on biostimulated soils(Obinna et al., 2015; Ukpaka, 2018).

3.4 Comparison of Model Performance for TPH Prediction

To demonstrate the predictability of the models, the TPH recorded from the experimental analysis for the treated and untreated wet and dry soils were compared with the first order rate kinetics and Michaelis-Menten model as shown in Figures 7 and 8.

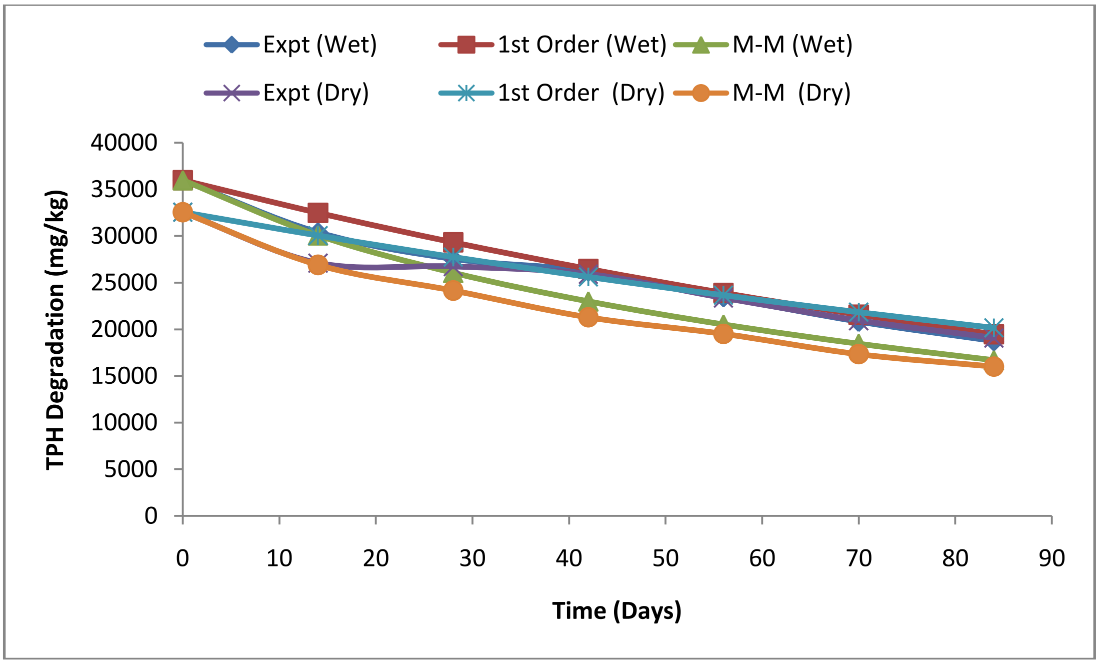

Figure 7: Comparison of predicted TPH concentrations for control samples

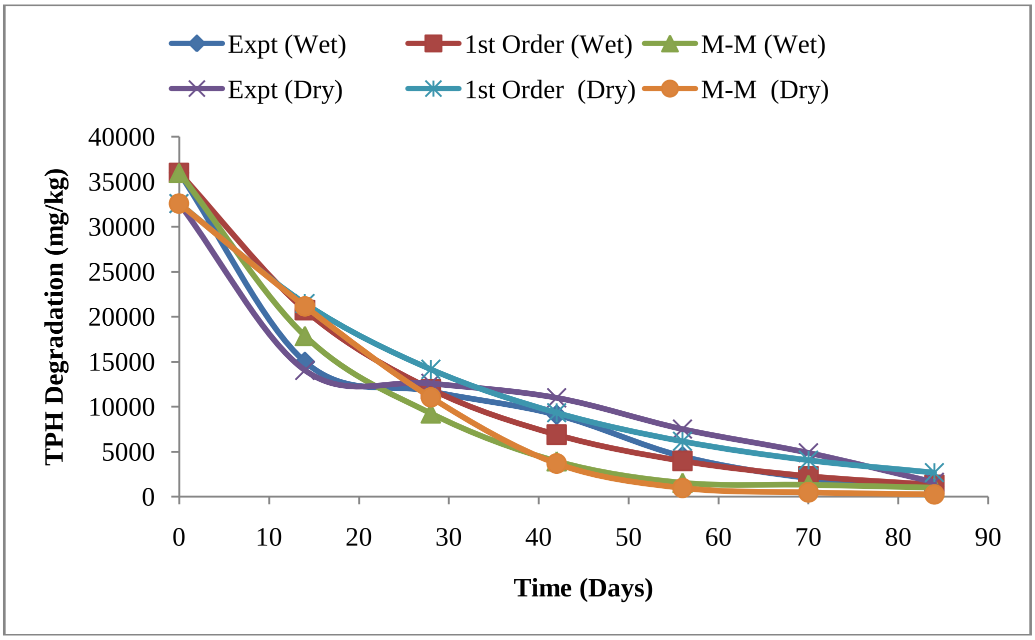

Figure 8: Comparison of predicted TPH concentrations at 50ml treatment

Figure 7 show the comparison of the first order and Michaelis-Menten Models with experimental results obtained from the control soil samples for wet and dry soils respectively. First order model showed a better fit with experimental data compared to Michaelis-Menten equation, as indicated by R^2 valuesfor first order degradation rate kinetics (0.9869 and 0.9529 for wet soil and dry soils) and Michaelis-Menten equation(0.8803 and 0.9484 for wet and dry soils). Similarly, as shown in Figure 8,the TPH predicted by the first order rate kinetics for both wet and dry soils fitted the experimental results than the Michaelis-Menten equation, indicating that the first order rate kinetics correlated better with experimental data than the Michaelis-Menten model. Hence, the obtained value of the correlation coefficient (R^2) from the first order rate was 0.9730 and 0.9033 for wet soil and dry soil, while for Michaelis-Menten model it was obtained as 0.9590 and 0.8318 for wet and dry soil. Tables 4 and 5 show the comparison of predicted and measured TPH for control and 50ml *Pseudomonas sp*. treated wet soil and dry soil samples.

Overall, the first order rate kinetics performed better than the Michaelis-Menten model. Hence, its extensive use in TPH degradation studies (Asgari*et al*., 2017; Aghalibe *et al*., 2017; Amagbo and Ere, 2019).

Table 4: Comparison of Model Prediction for Control Sample

Time (Days)	**Wet Soil**			**Dry Soil**		
	Expt.	1st Order	M-M	Expt.	1st Order	M-M
0	35981.1	35981.1	35981.1	32563.4	32563.4	32563.4
14	30389.6	32485.5	30075.7	27123.2	30065.8	26915.6
28	27547.1	29329.5	26065.2	26746	27759.8	24168.5
42	26233.8	26480.1	23000.3	25929.3	25630.6	21299.2
56	23463.3	23907.5	20525.1	23367.9	23664.8	19528.7
70	20825.9	21584.9	18460	20954.1	21849.7	17346.2
84	18746.1	19487.9	16699.3	19090	20173.9	16021.4

Table 5: Comparison of Model Prediction for Sample with 50ml Pseudomonas

Time (Days)	Wet Soil			Dry Soil		
	Expt.	1st Order	M-M	Expt.	1st Order	M-M
0	35981.1	35981.1	35981.1	32563.4	32563.4	32563.4
14	14968.1	20726.1	17843.6	14031.6	21455.7	21139.2
28	11697.5	11938.8	9245.04	12579.2	14136.9	11024
42	9085.22	6877.07	3919.68	10973.9	9314.65	3669.04
56	4461.65	3961.38	1534.29	7499.35	6137.32	954.847
70	2050.92	2281.87	1307.56	4864.97	4043.82	461.169
84	1090.23	1314.42	986.968	1595.61	2664.43	240.451

Conclusion

The study demonstrated that inoculation with *Pseudomonas sp*. significantly enhanced TPH degradation in crude oil contaminated wet and dry soils. Kinetic evaluation showed increase in first order rate constant and Michaelis-Menten parameters with microbial treatment. First order model provided more accurate TPH predictions compared to Michaelis-Menten model. Therefore, the estimated degradation rate constants can be effectively utilized for predicting TPH reduction during bioremediation of petroleum hydrocarbons contaminated soils.

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