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Modeling the Bioremediation of Diesel Contaminated Soil by Bacteria from Clogged Drainage System

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Abstract: The contamination of soil with petroleum products is a major environmental issue. The widespread application of diesel in human activities makes it one of the most hazardous petroleum products. Among the available remediation methods, bioremediation has become the main choice for petroleum products contaminated site recovery due to its cost-effectiveness and environmental-friendliness. In this study, the bioremediation of diesel contaminated soil by bacteria from clogged drainage was examined and modeled. Soil samples were contaminated with diesel and inoculated with cultured bacteria isolated from clogged drainage systems for 56 days. Experimental results indicated that *Pseudomonas, Micrococcus, Acinetobacter, Bacillus cereus* and *Providencia* species actively participated in the bioremediation process. The percentage reduction of diesel was statistically highly significant ($p<0.05$) for all five bacterial species and found to be in the following order: *Pseudomonas* (92.39%) > *Acinetobacter* (88.29%) > *Bacillus cereus* (88.11%) > *Micrococcus* (86.91%) > *Providencia* (29.64%). The biodegradation data complied with first-order kinetic model. Thus, first-order kinetic models of the biodegradation of diesel in soil for the five active bacteria were developed. The models were used to fit the biodegradation of diesel in soil with correlation coefficient (R^2) range of 0.8142 – 0.9599 and p-value range of 0.2879 – 0.8211 ($p>0.05$), indicating good agreement between the measured and predicted biodegradation of diesel in soil. Therefore, it is concluded that the developed models can adequately predict the biodegradation of diesel in soil with time by the respective bacteria.

Keywords: Modeling, Bacteria, Biodegradation, Clogged Drainage Systems, Bioremediation, Diesel, Contaminated Soil

1. Introduction

Diesel is a mixture of hydrocarbons produced from various sources, the most common being petroleum, while other sources include biomass, animal fat, biogas, natural gas and coal liquefaction [1]. It is made up of low molecular weight alkanes and polycyclic aromatic hydrocarbons [2]. According to U.S. Energy Information Administration [1], most of the products used in the world today are transported by trucks and trains with diesel engines, and most construction, farming and military vehicles and equipment also have diesel engines. Diesel is also used in diesel engine generators to generate electricity. As a transportation fuel, diesel offers a wide range of performance, efficiency, and safety features. It has a greater energy density than other liquid fuels, so it provides more useful energy per unit of volume. In 2018, diesel accounted for about 20% of total U.S. petroleum consumption by the transportation sector.

The contamination of soil with petroleum products is a major environmental issue. Soil may become contaminated with these hydrocarbons by various routes, such as leakage from underground storage tanks and pipelines, accidental spills during transportation, drilling sites and improper waste disposal practices. The presence of petroleum hydrocarbons in soils poses a huge threat to human health and natural ecosystems. The hydrocarbon composition of diesel makes it toxic to the environment and its widespread application in human activity makes diesel one of the most hazardous hydrocarbon pollutants [3]. Studies have shown that the contamination of soil by hydrocarbons have caused significant alterations on soil biological, chemical and physical properties, including increased soil water repellency [4-6]. According to Doerr et al. [7], soil water repellency affects hydrological and ecological soil functions by

decreasing water infiltration, increasing surface runoff and erosion, and impeding plant growth.

At present, there are several methods aimed at minimizing the negative effects of soil contamination. Bioremediation is considered to be a cost-effective and environmental-friendly approach for decontamination of polluted soils [8-10]. According to the Environmental Protection Agency, bioremediation is a water and soil treatment technique using naturally occurring microorganisms to attack hazardous materials and change them into less toxic substances. These naturally occurring microorganisms are nature's helpers in decomposing, recycling and rectifying imbalanced chemical conditions in soil and water [11]. Where the right microbes are low in numbers or entirely absent, bioremediation is introduced by adding amendments i.e. microbial actors like fungi and aerobic bacteria. This simple process is called bioaugmentation, and it's highly effective to correct conditions quickly, as long as the right environmental conditions are present [11]. Critical conditions for bioremediation include: (1) contaminants that provide fuel and energy to microbes, (2) microbes that feed on the contaminants and destroy them, (3) oxygen in sufficient amounts to support aerobic biodegradation (about 2% in the gas phase or 0.4 mg/liter in the soil or water), (4) soil moisture within 50 – 70% of the water holding capacity of the soil, (5) right temperature i.e. not too cold or hot, between 0 – 40°C (6) nutrients like nitrogen, phosphorous, potassium and sulfur to support microbe growth, and (7) pH in the range of 6.5 to 7.5 [10-11].

Bioremediation has become the main choice for contaminated site recovery in United States, and it's commonly used around the world for all sorts of situations of contamination [10-11]. The biggest benefit from using bioremediation processes is that it uses nature to fix nature. When properly applied, bioremediation is the safest and least invasive soil and groundwater cleanup available [10-11]. Bioremediation is effective for cleaning petroleum hydrocarbons and many other contaminants like polycyclic aromatic hydrocarbons, polychlorinated biphenyls, arsenic, fluoride, nitrate, metals, ammonia, phosphates, insecticides and herbicides, etc., and can be carried out on the site of contamination [10-11].

Drainage systems are provided to remove domestic wastewater and storm water in order to prevent flooding. Majority of drainage systems in urban cities of developing countries are poorly designed, constructed and managed leading to complete failure or being clogged. Stagnant water in clogged drains can become a breeding ground for harmful bacteria which in turn can lead to diseases like cholera, gastroenteritis and typhoid [12]. However, the bacteria in clogged drains can find useful application in bioremediation of petroleum hydrocarbon contaminated soil. Studies on the remediation of diesel contaminated soil using bacteria from clogged drains have been carried out however without modeling of the process [13]. Thus, the aim of this study is to use bacteria from a clogged drainage system to remediate diesel contaminated soil and then model the bioremediation process.

2. Materials and Methods

2.1. Contaminant

Automotive gas oil (AGO) popularly called diesel was used in this study. The diesel was bought from Londa fuel station at Bori. The diesel was first analysed in the laboratory to determine its total petroleum hydrocarbon (TPH) content.

2.2. Acquisition of Bacteria from Clogged Drainage System

The acquisition of the bacteria used in this study from clogged drainage system was fully described in our previous work [13]. But briefly, wet sediment samples were collected from clogged drainage systems at selected locations using a Grab Sampler. The sampling containers were sterilized to maintain interstitial microbial quality in the samples. Collected samples were labelled and stored in a cooler with ice packs prior to laboratory for analysis.

2.3. Identification of Bacteria in Clogged Drainage

The identification of the bacteria found in the clogged drainage system was fully described in our previous work [13]. The identification involved bacterial culture, isolation of bacteria colonies, and biochemical tests which include catalase test, methyl red test, oxidase test, indole test, and citrate test. The number of bacterial colonies in the clogged drainage sample was determined using spectrophotometer. Heterotrophic count of bacterial species was carried out to determine the number of bacterial cells in the clogged drainage sample. The bacterial growth process in the cultured solution was used to plot the bacterial growth with time.

2.4. Collection of Soil Samples

Loamy soil commonly found in most farmland was used for the experiment. Samples of loamy soil were collected from a depth of 0 to 60cm using a standard auger. Collected soil samples were analysed for indigenous bacteria, hydrocarbon content, pH and temperature to establish baseline condition before use in the experiment. Soil samples were heated at 1200°C to destroy the indigenous bacteria before use in the experiment.

2.5. Experimental Setup for Bioremediation of Diesel Contaminated Soil

A 10g soil sample was mixed with 1ml of diesel to provide known quantity of the soil sample and volume of the contaminant as well as the TPH. The contaminated soil sample was thoroughly mixed to ensure that the contaminant was evenly distributed in the soil sample. The experiment was set up in glass beaker. Eleven (11) experiments were set up, one for each bacterium. One (1) control was also setup, given a total of 12 setups for the study. Isolated bacteria solution (9ml) from the clogged drainage systems were inoculated into the 11 experimental setups while no

inoculation was done to the control. 0.5g of nitrogen and 0.5g of phosphorus were mixed in 100ml of distil water and 1ml was transferred into each sample to provide nutrients for the bacteria. The 12 setups were monitored for a period of eight (8) weeks and samples were taken from each on a weekly basis and analysed for TPH contents.

2.6. Data Analysis and Model Development

Analysis of variance (ANOVA) was performed to determine the significant level of biodegradation by each active bacterium isolates. The result was used to evaluate the biodegradation activities and their respective kinetics of reactions in the first-order kinetic models. A linear regression analysis was used to fit the biodegradation data into models. The bioremediation process was modeled using Monod and Michaelis-Menten modeling approach [14-15]. The model was developed based on the bacteria specific growth rate and yields shown in Equations (1) and (2).

$$\mu = \frac{\mu_{\max} S}{K_s + S} \quad (1)$$

where μ_{max} is the maximum specific growth rate, S is the substrate, and K_s is the substrate saturation constant.

$$r(C) = \frac{R_{\max} C}{K_m + C} \quad (2)$$

where r is the bacteria growth rate, R_{max} is the maximum growth rate, C is the concentration of nutrient, and K_m is the nutrient saturation constant.

The percentage of TPH biodegraded was computed using Equation (3).

$$\theta_R = \frac{TPH_0 - TPH_t}{TPH_0} \times 100 \quad (3)$$

where TPH_0 is the initial concentration of petroleum product in soil and TPH_t is the residual concentration of petroleum product in soil at time t.

3. Results and Discussion

3.1. Biodegradation of Diesel by Bacterial Species

While a total of eleven (11) bacterial species (*Bacillus cereus, Micrococcus, Staphylococcus, Salmonella, Escherichia coli, Proteus, Vibrio cholerae, Shigella, Providencia, Acinetobacter and Pseudomonas*) were identified from the clogged drainage wet sediment samples, only five (5) bacterial species (*Bacillus cereus, Micrococcus, Providencia, Acinetobacter and Pseudomonas*) actively participated in the bioremediation process (Figure 1). The existence of these bacterial species in clogged drainage wet sediment samples have been reported in the literature [13, 16-17]. Temperature and pH had a marked effect on the rate of diesel biodegradation. Effective biodegradation occurred in a neutral to slightly alkaline condition (pH 7.0 – 8.0) and temperature range of 27°C to 29°C. The maximum TPH removal occurred at pH 8.0 and temperature 29°C. Similar findings had been reported in the literature [18-20].

The result of the bioremediation of diesel contaminated soil by the five active bacteria isolates for the 56 days remediation period is showed in Figure 2. The percentage reduction of TPH on day 56 was statistically highly significant ($p<0.05$) for all five bacterial species and found to be in the following order *Pseudomonas* > *Acinetobacter* > *Bacillus cereus* > *Micrococcus* > *Providencia* (Table 1). This observation compares favourably well with the findings of some similar previous studies [20-21].

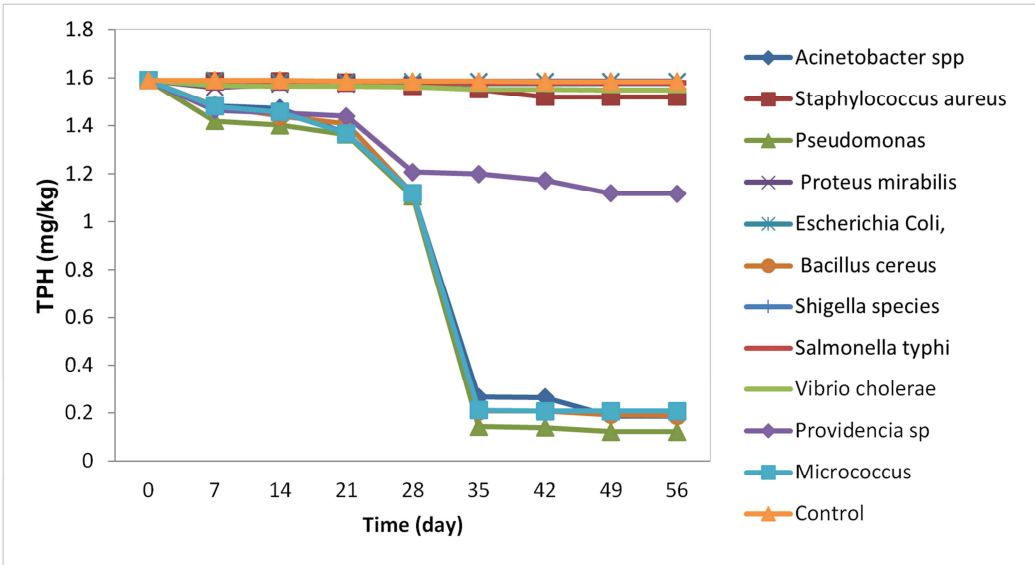

Figure 1. *Biodegradation of diesel in soil by all bacteria isolates.*

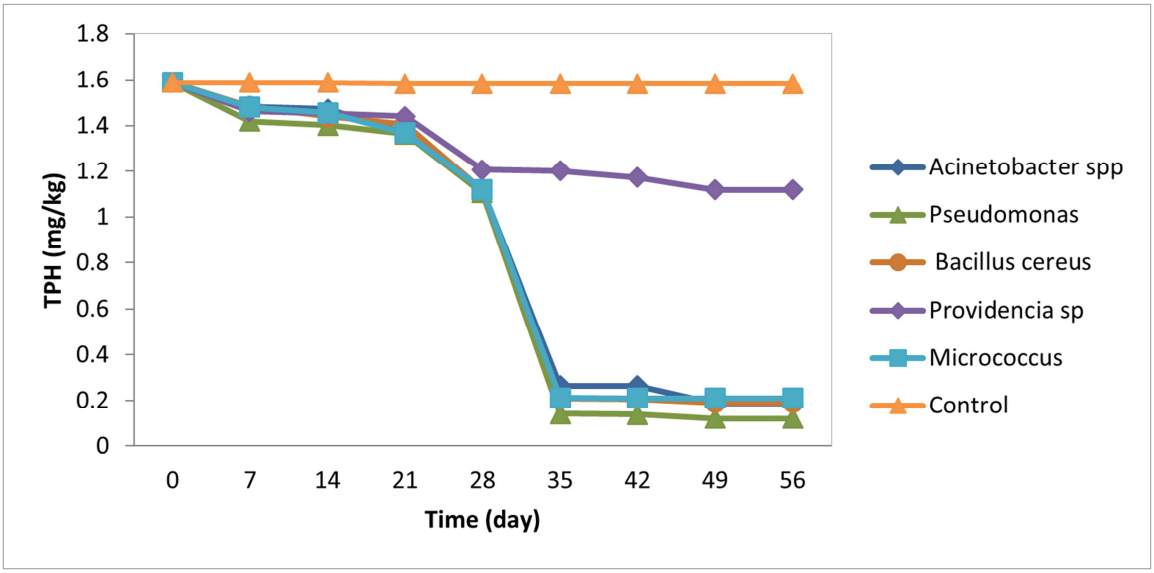

Figure 2. *Biodegradation of diesel in soil by active bacteria isolates.*

Table 1. *Percentage biodegradation of diesel in soil by active bacteria isolates.*

Bacteria type	Percentage degradation (%)	P-value	Level of significance
Pseudomonas	92.39	0.003	Significant
Acinetobacter	88.29	0.004	Significant
Bacillus cereus	88.11	0.004	Significant
Micrococcus	86.91	0.004	Significant
Providencia	29.64	0.002	Significant

3.2. Modeling of the Bioremediation Process

The biodegradation of diesel in the diesel contaminated soil sample followed first-order kinetics like what was reported in previous studies [21]. Microbial decomposition of petroleum hydrocarbon products can be represented using Equation (4). Thus, the bacteria degraded the diesel into carbon dioxide, water and energy [19-23].

$$C_n H_n + O_2 \xrightarrow[\text{Bacteria}]{} CO_2 + H_2O + \text{Energy} \quad (4)$$

The kinetic relationship between the reaction rate and the rate of change of TPH with time can be described using Equation (5). This describes the rate of TPH reduction in the soil.

$$r = \frac{\Delta TPH}{\Delta t} \quad (5)$$

where r is the rate of reaction, t is the time in day, ΔTPH is the change in TPH concentrations.

The order of kinetic reaction can be determined by written Equation (5) as:

$$r = \frac{dC}{dt} = -kC^n \quad (6)$$

where C is the concentration of TPH (mg/kg) remaining at any time (t), n is the reaction order, and k is the kinetic rate constant.

For first-order kinetic reaction, n = 1. Thus, Equation (6) becomes:

$$\frac{dC}{dt} = -kC \quad (7)$$

Kinetically, the biodegradation of substrate can be described by Monod's model [24].

$$\mu = \frac{\mu_{max} S}{K_s + S} \quad (8)$$

where μ_{max} is the maximum specific growth rate, S is the substrate, and K_s is the saturation constant or Monod's constant.

However, the Monod's Equation has been found not to precisely predict the bacterial growth and substrate utilization in a slow degradation and limited bioavailability media such as soil [25]. Therefore, the biodegradation of the diesel was modeled according to Michaelis-Menten model. The kinetics of the bacterial reactions can be represented by the Michaelis-Menten Equation [26]:

$$r(C) = \frac{R_{max} C}{K_m + C} \quad (9)$$

where r(C) is the rate of biodegradation (day^{-1}), R_{max} is the maximum specific rate of biodegradation (day^{-1}), C is the

concentration of TPH in soil (mg/kg), and K_m is the Michaelis' constant.

At low TPH concentrations C <<< K_m, the value of C in the denominator of Equation (9) is negligible compared with K_m and Equation (9) reduces approximately to:

$$r(C) = \frac{R_{\max}}{K_m} C \tag{10}$$

The ratio of constants R_{max}/K_m is the first-order rate coefficient for the biodegradation reaction. If $k_1 = R_{max}/K_m$, Equation (10) becomes:

$$r(C) = k_1 C \tag{11}$$

where k_1 is the half-saturation constant. Taking the derivative of Equation (11) with respect to time (t) gives:

$$\frac{dC}{dt} = -k_1 C \tag{12}$$

$$\frac{dC}{C} = -k_1 dt \tag{13}$$

where C is the concentration of TPH remaining in the soil samples at time t and k_1 is the first-order rate constant. Integrating Equation (13) with initial conditions, t = 0, t = t; C = 0, C = C_t gives:

$$\int_{C_0}^{C_t} \frac{dC}{C} = -k_1 \int_0^t dt$$

$$\ln(C_t) - \ln(C_0) = -k_1 t$$

$$\ln(C_t) = \ln(C_0) - k_1 t \tag{14}$$

The degradation of TPH in the soil with time can thus be expressed from Equation (14) as:

$$\ln(TPH_t) = \ln(TPH_0) - k_1 t \tag{15}$$

where TPH_t is the concentration of TPH (mg/kg) remaining at any time (t), TPH_0 is the initial concentration of TPH (mg/kg), and k_1 is the first-order reaction rate.

Equation (15) can be expressed as follows:

$$\ln\left(\frac{TPH_t}{TPH_0}\right) = -k_1 t \tag{16}$$

Equation (16) can further be expressed as Equations (17) and (18):

$$\frac{TPH_t}{TPH_0} = e^{-k_1 t} \tag{17}$$

$$TPH_t = TPH_0 e^{-k_1 t} \tag{18}$$

3.3. First-Order Half-Life

Half-life ($t_{1/2}$) is the time required for a contaminant to be degraded to one-half of the original concentration. For a first-order reaction, the half-life is computed from Equation (16) as follows:

$$t = \frac{\ln\left(\frac{TPH_t}{TPH_0}\right)}{-k_1} \tag{19}$$

For $t_{1/2}$, $TPH_t = 1/2TPH_0$

$$t_{1/2} = \frac{\ln\left(\frac{1/2TPH_0}{TPH_0}\right)}{-k_1}$$

$$t_{1/2} = \frac{\ln(2)}{k_1} \tag{20}$$

3.4. Kinetics of Bioremediation of Diesel Contaminated Soil

Equation (16) was used to determine the rate of bioremediation by the active bacteria isolates shown in Figure 3. Linear regression analysis was used to obtain the linear plots presented in Figures 4 to 8, and coefficient of determination (R^2) values were generated (Table 2). The linear model fits show the order of the bioremediation rate or kinetics for each bacterium. The high values of the coefficient of determination ($R^2 > 0.8$) indicate a good fit of the biodegradation data to the first-order kinetic models for the hydrocarbon utilization bacteria (HUB). Figures 4 to 8 illustrate that the bioremediation rates of diesel removal complied with first-order kinetics. The slope of the plot is the first-order kinetic constant (k_1) of Equation (16). This observation agrees with previous studies [21, 27]. The values of the reaction rate constant show that *Pseudomonas* degraded the diesel in the soil more efficiently than the other bacteria (*Acinetobacter* > *Bacillus cereus* > *Micrococcus* > *Providencia*).

The biodegradation reaction order (n) for the HUB was obtained as the exponent of the reaction rate constant using Equation (6) and rounded up to a whole number following previous studies [21]. The value of n for all 5 bacteria rounded up to 1, indicating first-order kinetic. The values of reaction rate constant were substituted into Equation (18) to

obtain the first-order kinetic models. Thus, the bioremediation of diesel contaminated soil can be described using the models in Table 3. The diesel biodegradation half-life for each bacterium was computed using Equation (20). The kinetic parameters of the first-order degradation models (Table 3) show that the highest rate of diesel degradation occurred using *Pseudomonas* (k_1 = 0.0585 day^{-1}) with 92.39% removal efficiency and half-life of 12 days while the least occurred using *Providencia* (k_1 = 0.0067 day^{-1}) with 29.64% removal efficiency and half-life of 103 days.

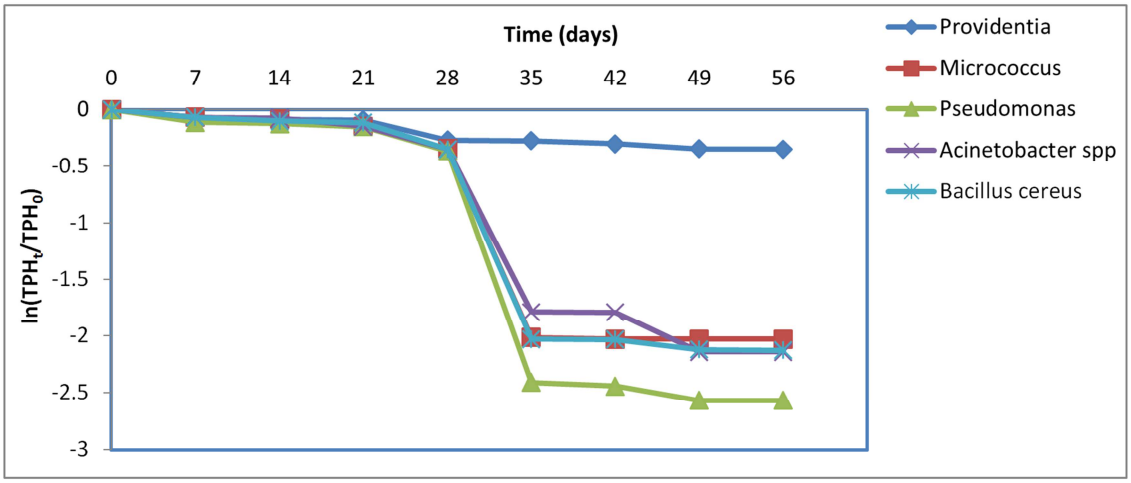

Figure 3. *Rate of bioremediation of diesel contaminated soil by different bacteria.*

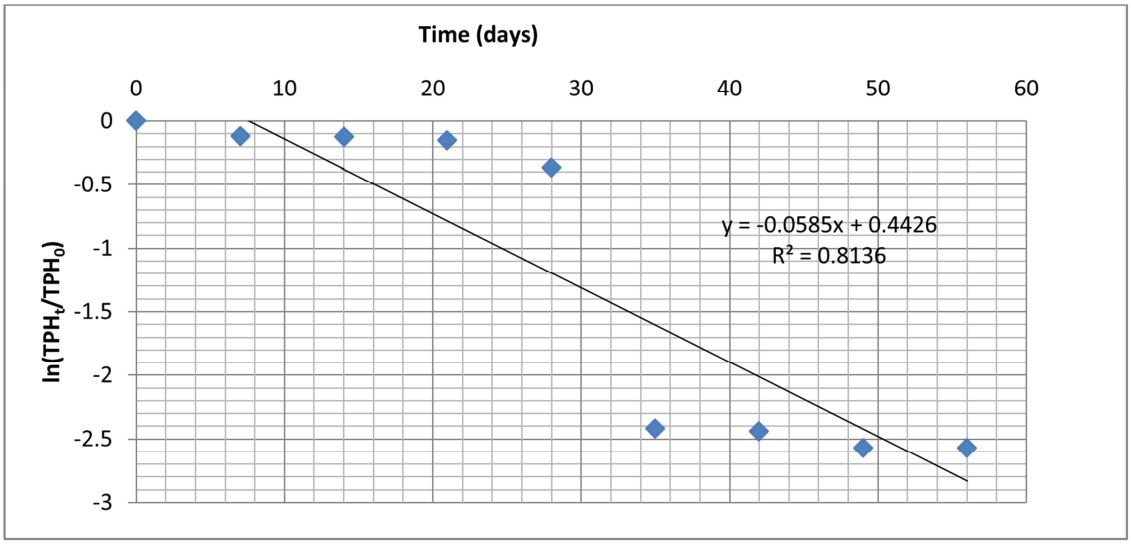

Figure 4. *First-order kinetic rate constant determination for Pseudomonas biodegradation of diesel.*

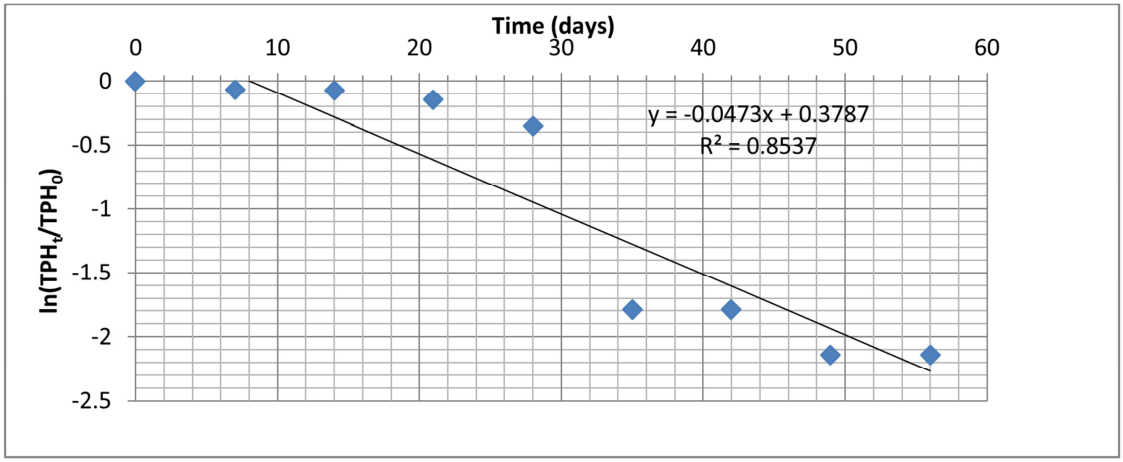

Figure 5. *First-order kinetic rate constant determination for Acinetobacter biodegradation of diesel.*

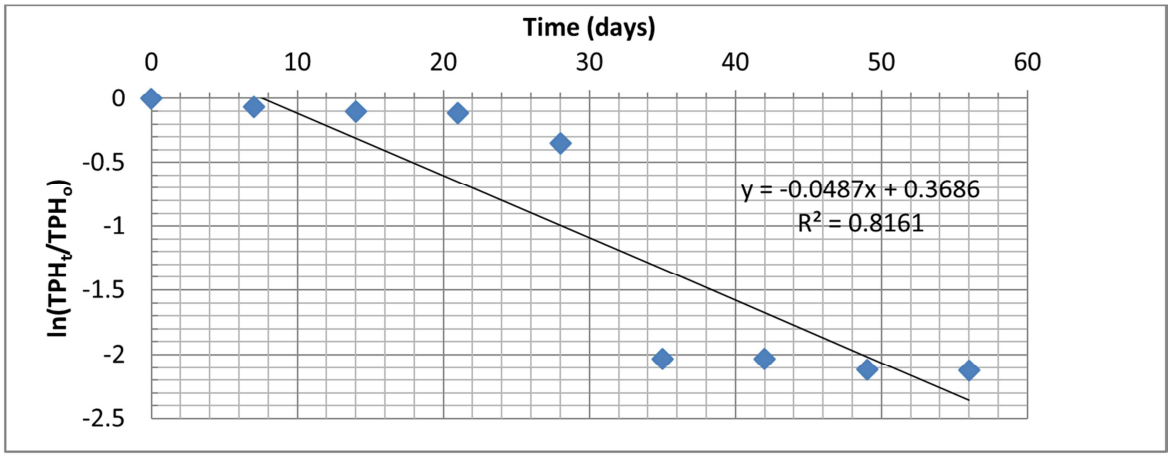

Figure 6. *First-order kinetic rate constant determination for Bacillus cereus biodegradation of diesel.*

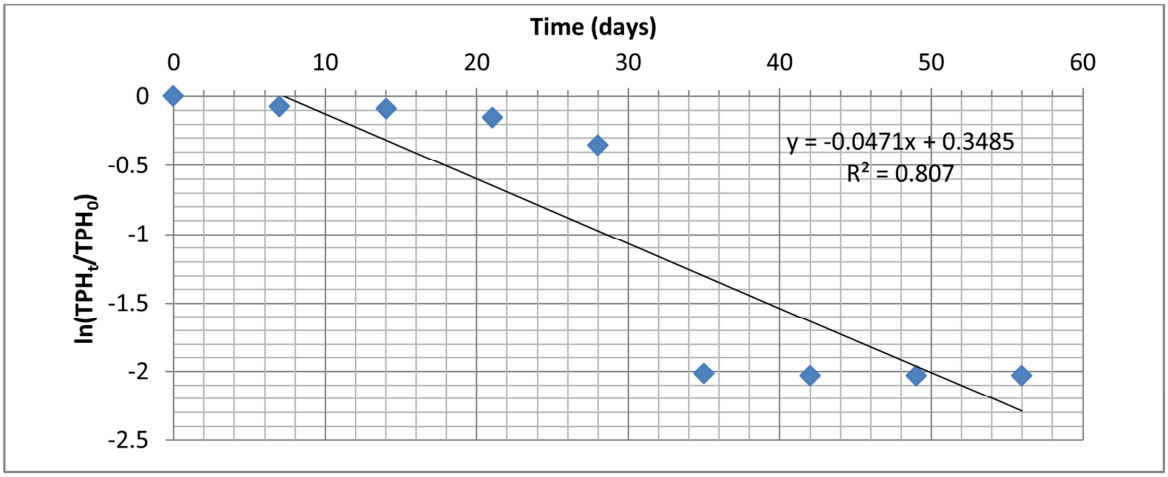

Figure 7. *First-order kinetic rate constant determination for Micrococcus biodegradation of diesel.*

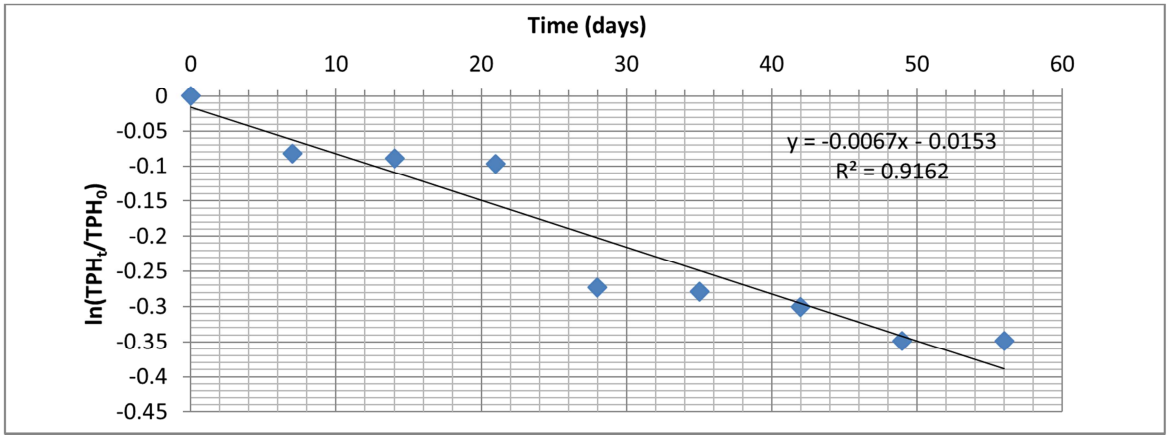

Figure 8. *First-order kinetic rate constant determination for Providencia biodegradation of diesel.*

Table 2. *Summary of biodegradation kinetics parameters for diesel.*

Bacteria type	**Kinetic parameters**		
	Biodegradation reaction order (n)	**k_1 (day^{-1})**	**R^2**
Pseudomonas	1.060	0.0585	0.814
Acinetobacter	1.040	0.0473	0.854
Bacillus cereus	1.050	0.0487	0.816
Micrococcus	1.048	0.0471	0.807
Providencia	1.007	0.0067	0.916

Table 3. *Summary of bioremediation prediction models and half-life for diesel contaminated soil.*

Bacteria type	First-order kinetic models	Half-life, $t_{1/2}$ (days)
Pseudomonas	$C_t = 1.589e^{-0.0585t}$	12
Acinetobacter	$C_t = 1.589e^{-0.0473t}$	15
Bacillus cereus	$C_t = 1.589e^{-0.0487t}$	14
Micrococcus	$C_t = 1.589e^{-0.0471t}$	15
Providencia	$C_t = 1.589e^{-0.0067t}$	103

4. Diesel Biodegradation Model Validation

The validation of the biodegradation models for individual bacterium using the relationship between the measured and predicted TPH concentrations are presented in Figures 9 to 13. The high correlation coefficients ($R^2 > 0.8$) obtained in all cases indicate good agreement between the measured and predicted biodegradation of diesel in soil. More so, the p-values ($p > 0.05$) obtained in all cases imply that there is no significant difference between the measured and predicted diesel biodegradation. Generally, the prediction of biodegradation of diesel in soil by *Providencia* was found to have greater accuracy ($R^2 = 0.9597$ and $p = 0.8147$) than by *Pseudomonas, Acinetobacter, Bacillus cereus* and *Micrococcus* ($R^2 = 0.8142 – 0.8508$ and $p = 0.2879 – 0.3259$) whose first 28 days were not perfectly predicted probably due to exhibition of lag phase in diesel biodegradation.

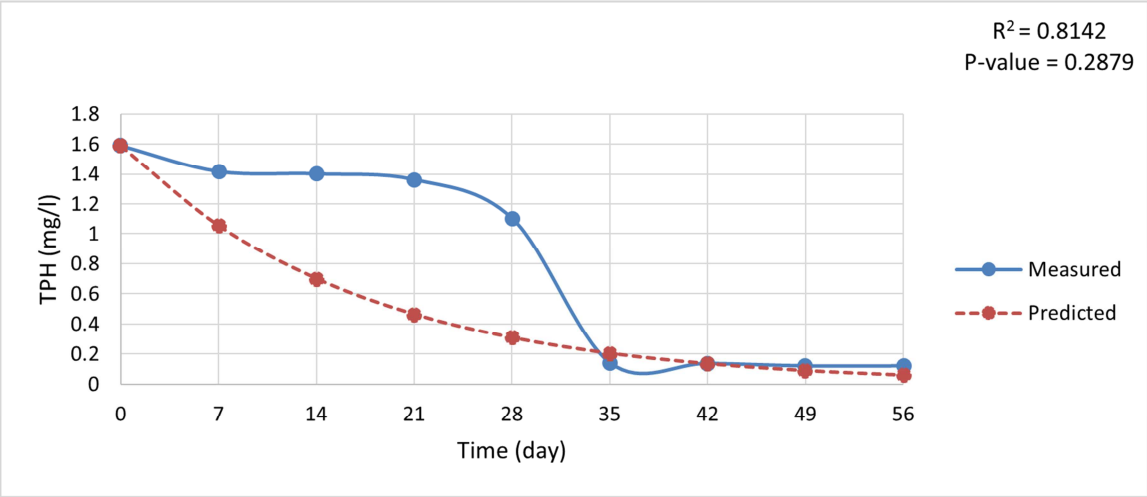

Figure 9. *Measured and predicted biodegradation of diesel by Pseudomonas.*

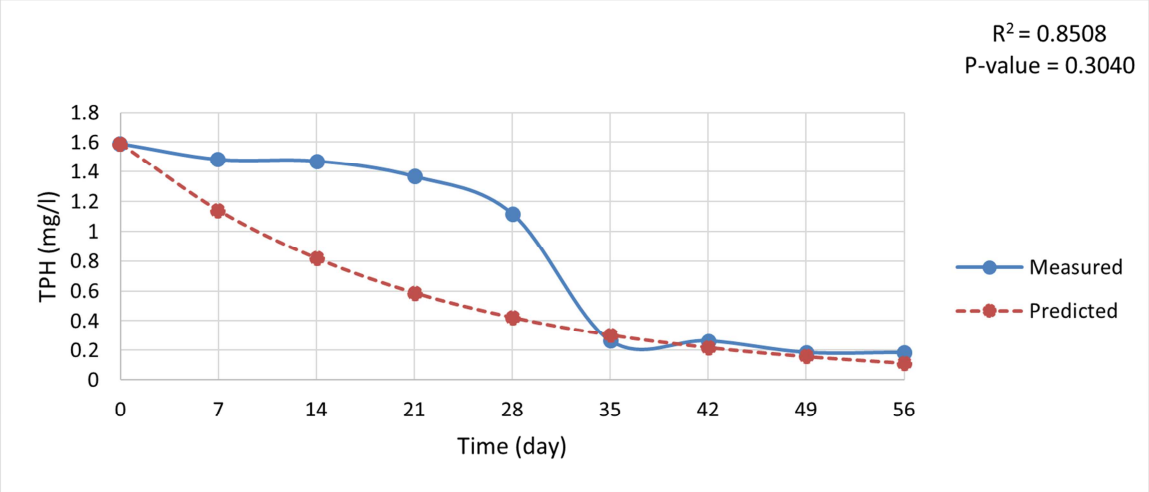

Figure 10. *Measured and predicted biodegradation of diesel by Acinetobacter.*

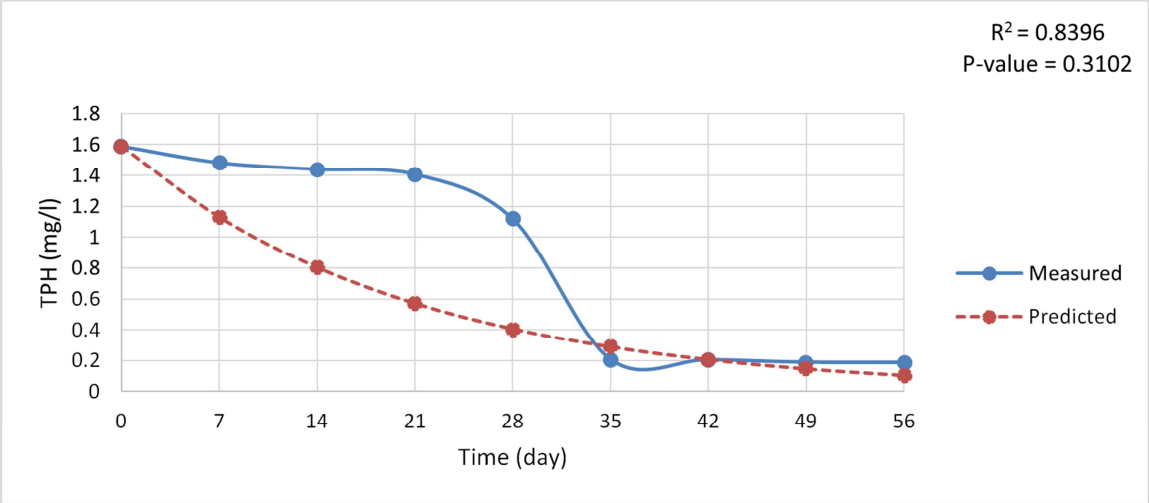

Figure 11. *Measured and predicted biodegradation of diesel by Bacillus cereus.*

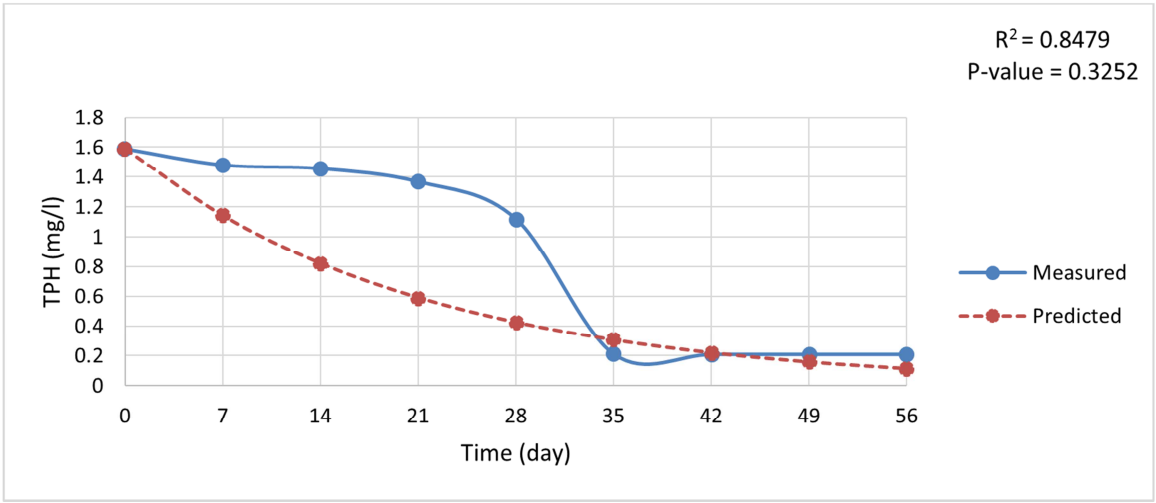

Figure 12. *Measured and predicted biodegradation of diesel by Micrococcus.*

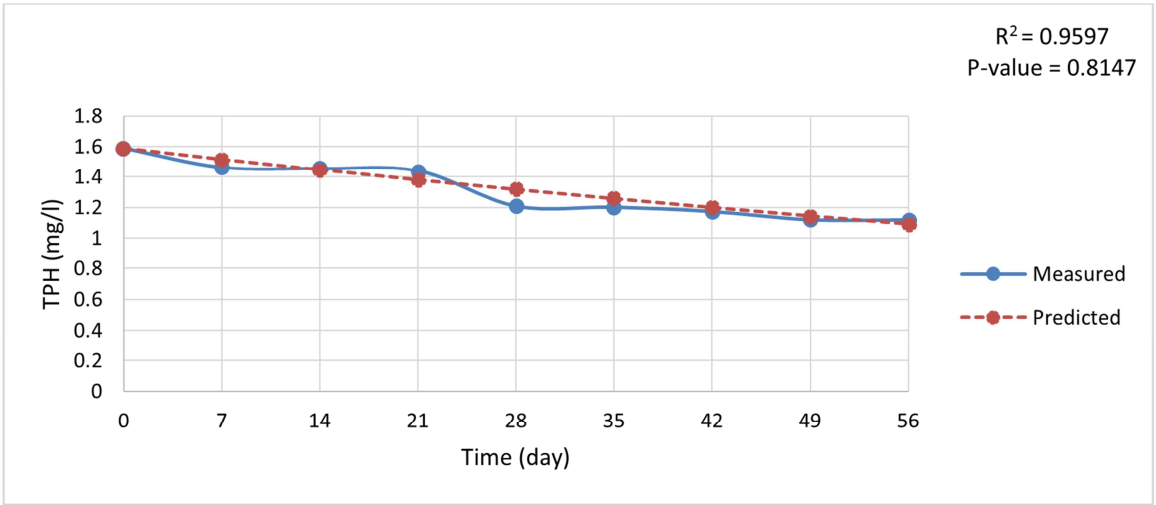

Figure 13. *Measured and predicted biodegradation of diesel by Providencia.*

5. Conclusion

The bioremediation of diesel contaminated soil using bacteria from clogged drainage systems was investigated and modeled. Five bacterial species (*Pseudomonas, Micrococcus, Acinetobacter, Bacillus cereus and Providencia*) actively

participated in the bioremediation process. The percentage reduction of diesel was statistically highly significant ($p<0.05$) for all five bacterial species and found to be in the following order: *Pseudomonas* (92.39%) > *Acinetobacter* (88.29%) > *Bacillus cereus* (88.11%) > *Micrococcus* (86.91%) > *Providencia* (29.64%). The biodegradation data complied with first-order kinetic model. Thus, first-order kinetic model of the biodegradation of diesel in soil for each of the five active bacteria was developed. The models were used to fit the biodegradation of diesel in soil with correlation coefficient (R^2) range of 0.8142 – 0.9597 and p-value range of 0.2879 – 0.8147 ($p>0.05$), indicating good agreement between the measured and predicted biodegradation of diesel in soil. Therefore, it is concluded that the developed models can adequately predict the biodegradation of diesel in soil with time by the respective bacteria.

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Conflicts of Interest

The author declared no conflicts of interest with respect to the research and publication of this article.

Availability of Data and Material

Not applicable.

Code Availability

Not applicable.

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