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Optimisation of Diesel Polluted Soil Using Response Surface Methodology

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Abstract: This research is based on the investigation of the bioremediation of diesel polluted soil using biostimulation strategy. The study was carried out on a diesel contaminated soil and concentrations: Tween 80 (5-15ml), Poultry droppings (20-60g), Hydrogen Peroxide (0.5-1.5ml) were added and incubated for 28 days of remediation period. Response Surface Methodology with Box-Behnken Design (BBD) was used with three factors and three levels of Tween 80, Poultry droppings and Hydrogen Peroxide as independent variables while diesel oil (Total Petroleum Hydrocarbon) removal was the dependent variable (response). The result showed disparities in the diesel oil biodegradation pattern with respect to Tween 80, Poultry droppings and Hydrogen Peroxide. The statistical analysis, via ANOVA showed coefficient of determination R^2 to be 99.89% and $P < 0.05$. The predicted optimum parameter of Tween 80: 10.10ml, Poultry droppings: 41.46g and Hydrogen Peroxide: 1.10ml were gotten while 56.565% was gotten as the optimal diesel oil removal. At this optimum condition, the observed diesel oil removal was found to be 56.568%. It can be concluded that bioremediation of diesel-contaminated soil resulted in petroleum hydrocarbon degradation.

Keywords: Bioremediation, Diesel, Tween 80, Soil

1. Introduction

Diesel is a fuel obtained from petroleum distillation and it is used in diesel engines. It is a lightweight mixture of liquid hydrocarbons and has a relatively low ignition temperature (540°C) and is ignited by the heat of compression. It is produced from the fractional distillation of crude oil between 200°C (392°F) and 350°C (662°F) at atmospheric pressure. When the soil becomes contaminated, the ecosystem is altered and agricultural activities are affected seriously due to this pollution. [5]; stated that used or improperly disposed diesel fuel contains potentially toxic substances, such as: benzene (carcinogens), lead, arsenic, zinc and cadmium, which can seep into the ground and contaminate ground water. Furthermore, it is common to observe diesel spills from the cleaning of oil tankers and also via the disposal of used oil from diesel cars by the road side mechanics. Physicochemical technologies have been previously used for the remediation of polluted soils [8].

According to [3]; bioremediation technology through the mechanism of biodegradation has been recognized to be a valuable alternative for the detoxification and disposal of toxic substances. This is because it is environment-friendly, financially viable, and technologically simple and it also conserves soil texture and characteristics.

Furthermore, bioremediation is an ecologically acceptable technology that is used for the removal of a pollutant from the biosphere through the use of micro-organisms [13]. It relies on biological processes to minimize unwanted environment impact of the pollutants. Nevertheless, this Research is focused on biostimulation method. According to [11]; biostimulation is the addition of nutrients to a polluted site in order to encourage the growth of naturally occurring chemical degrading microorganisms. This research is based on optimising diesel polluted soil.

2. Materials and Method

2.1. Method

Diesel-contaminated soil was excavated from the Generator House at Landmark University Cafeteria, Omu-Aran, Kwara State, Nigeria between 0-30cm from the soil surface. The soil was sieved with 5mm sieve. Organic wastes was used in this study instead of NPK fertilizer, to facilitate aeration through small pores to increase the water-holding capacity of the soil; thus enhancing bioremediation. The organic wastes used in this study was collected from Landmark University Research Poultry Farm. Physicochemical properties of organic wastes and soil employed were determined using standard methods.

2.2. Physicochemical Property Determination for Soil

Physicochemical properties determined for soil included pH, water holding capacity, textural class as well as Nitrogen, Potassium, Carbon and Phosphorus content.

2.3. Soil pH Determination

10 g of sieved ($\leq$ 5 mm) and air-dried soil was weighed poured into a 50ml beaker and 25 ml of distilled water added. The content was stirred manually for 30 minutes with a glass rod and left to stand for 1 hour. The electrode of the pH meter (Kent EIL 7055) was then inserted and the pH determined.

2.4. Determination of Water Holding Capacity (WHC)

Six (6) inches of soil was placed in percolation tube and compacted by gentle bouncing. Water was added until the water level reached 2 inches above soil level. The tube was covered and left to stand for 2 days. After this period, the top half-inch of soil was discarded and wet soil was weighed into a pre-weighed evaporating dish. The sample was placed in oven at 110°C for 24 h. It was then removed and weighed to obtain bone dry weight of the sample. Percentage water holding capacity was calculated as [12]:

$$\%waterholdingwater = \frac{wetweight-bonedryweight}{bonedryweight} \quad (1)$$

2.5. C, N, P and K Content

Soil textural class, as well as soil carbon, nitrogen, phosphorus and phosphorus contents were analyzed at the Civil Laboratory of Landmark University, Omu-Aran, Kwara state. Soil textural class was determined using the hydrometer method [7]. The organic carbon, phosphorus and nitrogen contents were determined using an element detector.

2.6. Bioremediation Setup

200g of diesel polluted soil was placed in plastic bottles, labeled 1 to 17. Organic wastes (poultry droppings), H_2O_2 and Tween 80 were added to each of the diesel polluted soil in appropriate conditions. The soil was then mixed daily to provide sufficient aeration and moistened by the addition of water every other day to adjust the water holding capacity at 60% throughput throughout the experimental period. The plastic bottles were then incubated at room temperature. The controls contained only diesel polluted soil.

2.7. Experimental Procedure

Polluted soil samples (200g) were placed in plastic bottles (microcosm). The diesel-contaminated soil in each plastic bottle was amended with different amounts of poultry dung (20 - 60g), Tween 80 (5 - 15 mg/l) and hydrogen peroxide (0.5 - 1.5 g/l), respectively. Soil used as control was not amended with any biostimulating agents. In total, 17 microcosms were settled and incubated for 28 days. All microcosms were mixed manually once per week to enhance oxygenation, and kept moist during the 28 day experimental period. Samples were withdrawn after the incubation period for percentage diesel removal analysis.

2.8. Experimental Design and Data Analysis

Table 1. Experimental range and the levels of the variables.

Factors	High level (+1)	Medium level (0)	Low level (-1)
Tween 80 (A) ml	5	10	15
Poultry Droppings (B)	20	40	60
Hydrogen Peroxide (C) ml	0.5	1.0	1.5

Table 2. Full-factorial Box-Behnken design for the three independent variables.

Run	Factor 1 A: Tween 80 (ml)		Factor 2 B: Poultry Droppings (g)		Factor C C: Hydrogen Peroxide (ml)	
	Coded	Uncoded	Coded	Uncoded	Coded	Uncoded
1	0	10.00	+1	60.00	-1	0.50
2	0	10.00	0	40.00	0	1.00
3	+1	15.00	0	40.00	-1	0.50
4	+1	15.00	0	40.00	+1	1.50
5	0	10.00	-1	20.00	+1	1.50
6	0	10.00	0	40.00	0	1.00
7	+1	15.00	-1	20.00	0	1.00
8	0	10.00	0	40.00	0	1.00
9	-1	5.00	-1	20.00	0	1.00
10	-1	5.00	0	40.00	-1	0.50
11	0	10.00	+1	60.00	+1	1.50
12	-1	5.00	0	40.00	+1	1.50
13	+1	15.00	+1	60.00	0	1.00
14	-1	5.00	+1	60.00	0	1.00
15	0	10.00	0	40.00	0	1.00
16	0	10.00	0	40.00	0	1.00
17	0	10.00	-1	20.00	-1	0.50
18 control	-		-		-	

The Box-Behnken factorial experimental design employed had three independent variables: Tween 80 (surfactant), Poultry droppings (nutrient) and hydrogen peroxide. Each of the independent variables was studied at three levels (1, 0, +1), with 17 experimental runs and one control. The levels were selected based on preliminary study results and literature. The variables optimized were Tween 80 (5 – 15

mg/l), Poultry droppings (20 − 60 g) and hydrogen peroxide (0.5 − 1.5 g/l) at three levels, respectively were shown in Table 1. Efficiency of diesel removal was assessed after 28 days. Table 2 shows the coded and actual values of factors and levels used in the experimental design. Diesel contaminated soil without biostimulation was also analyzed as a control. The statistical software Design Expert 6.0.8, (Stat-Ease Inc., Minneapolis, USA) was used to evaluate the analysis of variance (P < 0.05) to determine the significance of each term in the fitted equations and to estimate the goodness of fit in each case.

2.9. Total Petroleum Hydrocarbon Analysis

The TPH analysis was carried to determine the percentage diesel removed using the gravimetric method. 10g of each treatment was collected and dried at room temperature for 72 hours. 5g of the soil was placed in 200 milliliters beaker and 150ml of toluene was added. The mixture was stirred continuously for 30 min, left to stand in a fume cupboard for 2 hours and then filtered using Whatman No 42 filter paper. The residue, (soil), was allowed to dry in an oven at 50°C. The TPH was calculated according to [1]; [2]:

$$\% \text{ Diesel Removal} \left(\frac{g}{kg}\right) =$$

$$\frac{(\text{initial weight of soil}-\text{weight of soil after soil extraction})\text{in g}}{\text{initial weight of soil in kg}} \quad (2)$$

3. Results and Discussions

3.1. Soil Parameters

Table 3 showed the values for the soil parameters that were tested.

Table 3. Soil Parameters for the Diesel-polluted soil.

Parameters	Values
Soil Ph	7.29
Water holding capacity	16 ($\pm$2) %
Nitrogen content	0.34 mg/l
Potassium content	6.0 mg/l
Calcium content	26 mg/l
Phosphorus content	0.15mg/l

3.2. Bioremediation and Natural Attenuation

The experiment was designed using Response Surface Methodology, and after performing 17 experimental runs of the Box-Behnken design (BBD) and one control (see Table 2), the results of the statistical experiments were analyzed with reverence to the coded design matrix. The regression equation showed that Diesel degradation rate was an experimental function of test variables in coded units. The result from Table 4 showed that on day 28 (4th week), diesel content had decreased in all the soil microcosms.

In control, natural Bioattenuation removed 21.15% of petroleum hydrocarbons from the soil. It was observed that the respective reduction in petroleum hydrocarbon (TPH) of soil microcosms with amendments was much higher when compared to control in the same period in Table 4. This observation showed that the addition of biostimulants increased the rate of TPH degradation in the soil. This is in agreement with the report of [3] that an increase SEO biodegradation with the addition of biostimulants such as NPK, Tween 80 and Pig Manure as supplements. However, [9]; stated that one of the major factors limiting degradation of hydrocarbons is their low availability to the microbial cells [9]. In addition, hydrocarbon-oxidizing potential has also been shown to increase with hydrocarbon exposure. Thus, in Table 4, run numbers 12 and 13 (at lower concentration of Poultry droppings and H_2O_2), and run numbers 8 and 9 (at higher concentration of Poultry droppings and H_2O_2) had same remediation conditions but with different surfactant (Tween 80) concentration, results showed that addition of surfactant can enhance diesel degradation. Similar observations was made by [6] concerning the impact of biosurfactants on soil bioremediation.

The effect of different concentrations of hydrogen peroxide supplementation were investigated at the same condition of Poultry droppings and Tween 80 (run numbers 4 and 8, and run numbers 5 and 14) and the findings demonstrated that addition of hydrogen peroxide (H_2O_2) can enhance the bioremediation process of soil contaminated with diesel. This is in agreement with [4]. On the other hand; run numbers 11 and 12 and run numbers 5 and 7 through similar condition but with different amount of Poultry droppings were tested and the results showed that extra amount of Poultry droppings can improve diesel removal from contaminated soil.

Table 4. Experimental design and Results for bioremediation of Diesel polluted soil.

Run	Percentage of Diesel removal	
	Observed value	**Predicted value**
1	56.260	56.446
2	41.670	41.554
3	56.780	56.446
4	42.470	42.685
5	47.760	47.709
6	56.240	56.446
7	46.580	46.249
8	44.930	44.763
9	45.700	45.485
10	48.210	48.261
11	41.570	41.406
12	39.190	39.306
13	40.660	40.824
14	49.300	49.631
15	43.460	43.627
16	56.320	56.446
17	56.630	56.446
18 (control)	21.15	-

3.3. Second Order Polynomial Regression Model and Statistical Analysis

The experimental data were fitted to a second order polynomial regression model containing 3 linear, 3 quadratic and 3 interaction terms [10] using the same experimental design software to derive the Regression equation for diesel

removal from polluted soil as stated in eqn. 3:

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC \quad (3)$$

where β_0 is the value of the fixed response at the center point of the design; β_1, β_2, β_3 are linear coefficients; β_{12}, β_{13}, β_{23} are quadratic coefficients; are the interaction effect coefficients regression terms, respectively; A, B and C are the levels of independent variables. The significance of each coefficient in the equation was determined by F-test and P-values. F-test showed that all the factors and interactions considered in the experimental design are statistically significant i.e. $P < 0.05$, at 95% confidence level. The regression equation obtained after analysis of variance gave the level of diesel removal as a function of the different biostimulation variables: Tween 80, Poultry droppings, and Hydrogen peroxide.

The response (Y) generated is:

$$Y = 56.45 + 0.42A + 0.71B + 0.98C - 9.75A^2 - 5.93B^2 - 2.56C^2 - 0.34AB - 0.055AC - 0.023BC \quad (4)$$

Where A is Tween 80 concentration, B is Poultry Droppings concentration; C is Hydrogen peroxide concentrations. To test the fit of the model, the regression equation and determination coefficient (R^2) were evaluated. The model F-value of 717.44 infers the model is significant. The low probability value (<0.0001) indicates that the model is significant. Coefficient determination ($R^2 = 0.9989$) being a measure of goodness of fit to the model indicated a high degree of correlation between the observed value and predicted values. The determination coefficient ($R^2 = 0.9989$), suggested that more than 99.89% of the variance is attributable to the variables and indicated a high significance of the model. Thus, 0.11% of the total variance cannot be explained by the model. The fitted model was considered adequate if the F-test is significant ($P < 0.05$) [4]. The Analysis Of Variance (ANOVA) as shown in Table 5 demonstrated that the model was highly significant, as was apparent from the very low probability ($P < 0.0001$) of the F - test and insignificant result from the Lack of Fit model ($P = 0.1948$). The lack of fit test was implemented by comparing the variability of the current model residuals to the variability between observations at replicate settings of the factors. The Lack of Fit F-value of 2.54 implied the Lack of Fit is not significant relative to the pure error.

The Lack of Fit was designed to determine whether the selected model is adequate to describe the observed data, or whether a more complicated model should be used. The Predicted R-Squared value of 0.9881 is in reasonable agreement with the Adjusted R Squared value of 0.9975. Adequate Precision measures the signal to noise ratio. A ratio > 4 is desirable. The ratio of 71.064 obtained in this research indicates an adequate signal. This model can be used to navigate the design space.

The coefficient of variation (CV) as the ratio of the standard error of estimate to the mean value of the observed response is a measure of reproducibility of the model, generally a model can be considered reasonably reproducible if its CV is not greater than 10 per cent. Hence, the low variation Coefficient value (CV = 0.66 per cent) obtained indicates a high precision and reliability of the experiments. The coefficient of the model (parameter estimation) and the corresponding P-values are presented in Table 6.

The significant regression coefficients was considered, ignoring those with an insignificant effect on the response at a significance level of 95%. The P-values of the regression coefficients suggested that among the test variables, linear, quadratic and interaction effects of Tween 80, Poultry Droppings and hydrogen peroxide are highly significant. The insignificant effects (factors and interactions) with P-values higher than 0.05, were ignored. In this study, A, B, C, A^2, B^2, C^2, AB, AC and BC are significant model terms. Thus, statistical analysis of all the experimental data showed that NPK fertilizer, Tween 80 and hydrogen peroxide concentration had a substantial effect on diesel removal in this study.

Table 5. *Analysis of variance (ANOVA) for the quadratic response surface model fitting to the biodegradation data of diesel.*

Source	Sum of Squares	DF	Mean Square	F- value	P – value
Model	638.54	9	70.95	717.44	< 0.0001
Residual	0.69	7	0.099	-	-
Lack of Fit	0.45	3	0.15	2.54	0.1948
Pure Error	0.24	4	0.060	-	-
Correlation Total	639.23	16	-	-	-

Standard deviation = 0.31 C. V=0.66 R^2=0.9989.

Adjusted R^2 = 0.9975, Predicted R^2= 0.9881, Adequate Precision = 71.064.

Table 6. *Coefficient of the model for diesel biodegradation.*

Variables	Coefficient Estimate	Standard Error	F-value	P-value	Remarks
β_0	56.45	0.14	717.44	< 0.0001	Significant
β_1	0.42	0.11	14.02	0.0072	Significant
β_2	0.71	0.11	40.49	0.0004	Significant
β_3	0.98	0.11	78.29	< 0.0001	Significant
β_{11}	-9.75	0.15	4045.81	< 0.0001	Significant
β_{22}	-5.93	0.15	1494.94	< 0.0001	Significant
β_{33}	-2.56	0.15	278.60	< 0.0001	Significant
β_{12}	-0.34	0.16	4.74	0.0658	Significant
β_{13}	-0.055	0.16	0.12	0.7368	Significant
β_{23}	-0.023	0.16	0.020	0.8902	Significant

Figure 1 showed the studentized residuals and normal per cent probability plot. Residual showed the difference between the observed value of a response measurement and the value that is fitted under the theorized model. Small residual values indicated that model prediction is accurate.

The predicted versus actual plot of diesel oil biodegradation were shown in Figure 2. Actual values were determined for a particular run and the predicted values were calculated from the approximating function used for the model. The Cooks distance and studentized residuals illustrated the normal distribution and constant variance of the residuals, the goodness of fit, linearity of the fitted model, and the independence. Figure 2c showed Cook's distance plot; according to this plot there were no points that were potentially powerful due to their location in the factor.
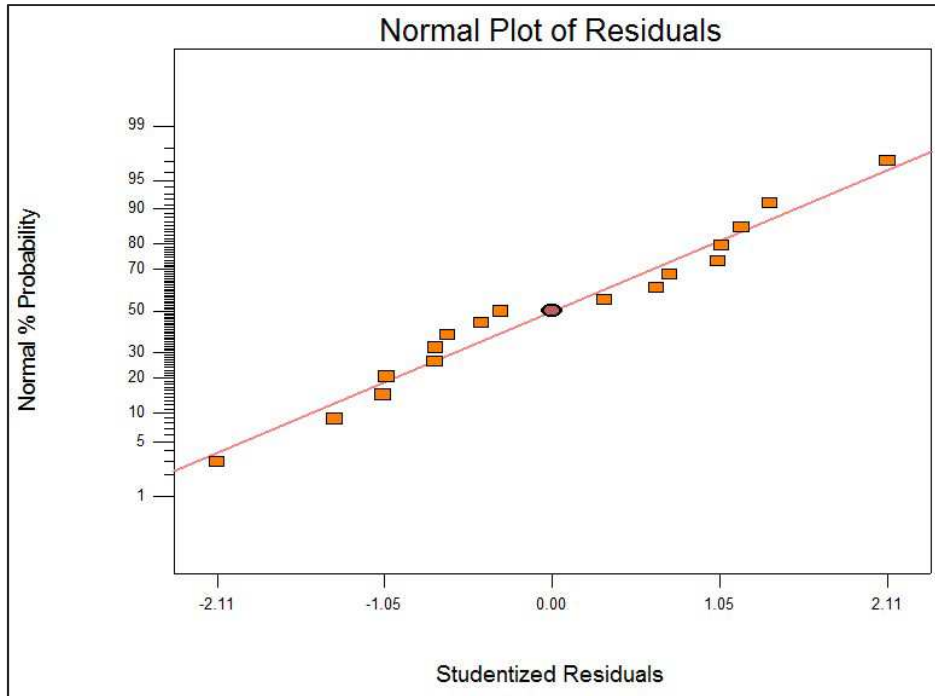
Figure 1. *Normal plot of residuals plot of soil diesel bioremediation.*
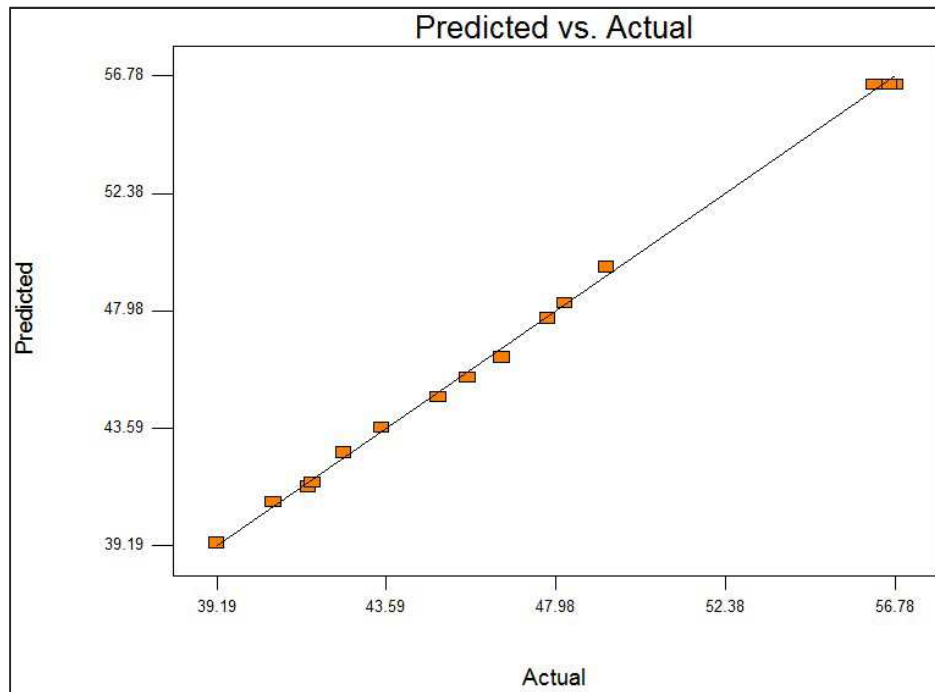
Figure 2. *Predicted versus actual plot of soil diesel bioremediation.*
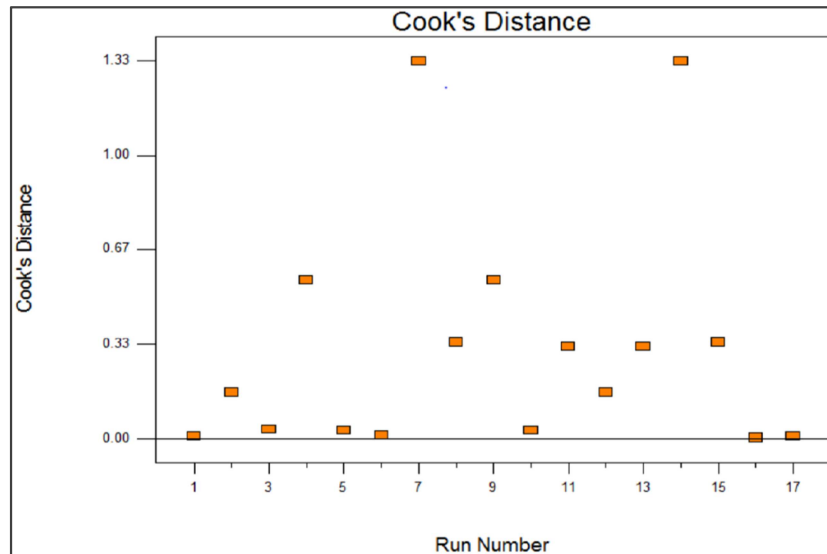
Figure 3. *Cook's distance plot of soil diesel bioremediation.*

3.4. Interaction Among Factors That Influence Diesel Oil Removal

The graphical representation of the response shown in Figures 4– 6 showed the effect of Tween 80 (A), Poultry droppings (B) and hydrogen peroxide (C) on removal of diesel. The effect of the interaction of poultry droppings and Tween 80 on diesel bioremediation is illustrated in Figure 4. It was observed in this study that; higher rate of diesel removal was attained with higher surfactant (Tween 80) concentration and relatively high amount of Poultry droppings. The maximum degradation yield of diesel (56.5652%) was obtained with 10.10ml of Tween 80 surfactant and 41.47g of Poultry droppings at a fixed hydrogen peroxide concentration of 1.10 ml. This was because of better bioavailability of substrate for the inherent microorganisms.

Figure 5 shows the 3D response surface plot of the interaction effect between Tween 80 and hydrogen peroxide (H_2O_2) concentrations. This plot demonstrated that both Tween 80 and hydrogen peroxide have the best interaction performance at optimum concentrations. A higher percent diesel oil removal was obtained at a higher hydrogen peroxide concentration with relatively high amount of tween 80. This three dimensional plot explained that both tween 80 and hydrogen peroxide have individual impact on diesel oil removal as the individual coefficient of both Tween 80 and hydrogen peroxide is positive and their interaction effect is positive. Figure 4.6 showed the response surface 3D plot of the effect of interaction between Poultry droppings and hydrogen peroxide concentrations. Higher rate of diesel oil removal was observed with increase in hydrogen peroxide and poultry droppings concentration due to positive interaction effect. Due to dominating interaction effects of hydrogen peroxide, higher levels of this variable gave higher yields of diesel oil removal.
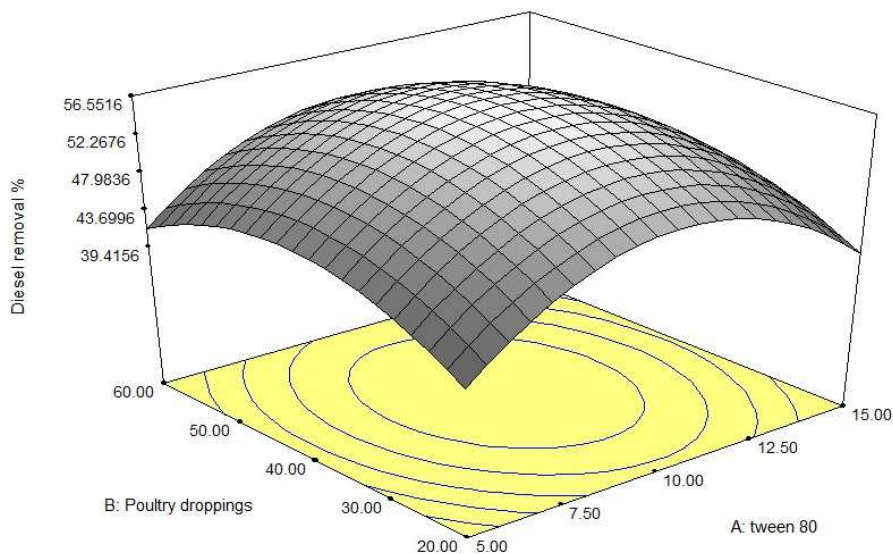

Figure 4. *Response surface 3D plots indicating interaction effects of factors Tween 80 and Poultry droppings.*
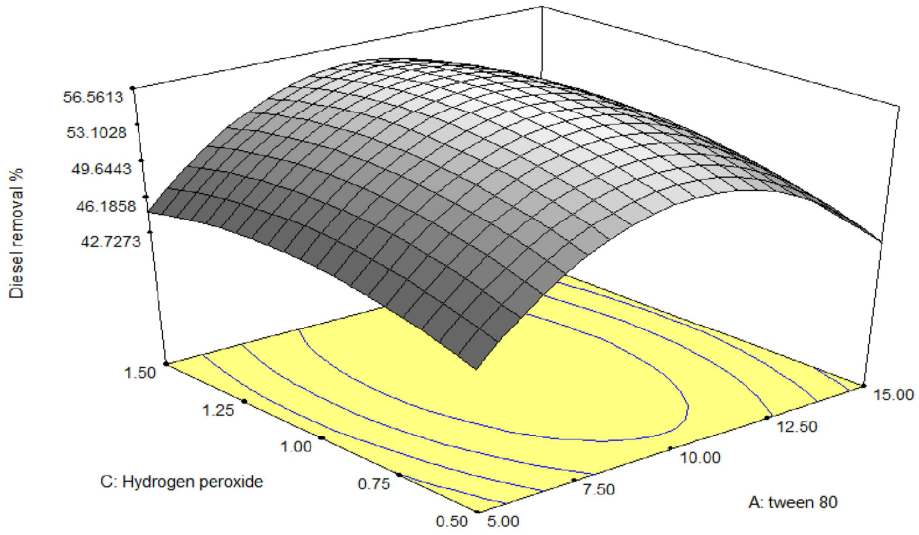
Figure 5. *Response surface 3D plots indicating interaction effects of factors Tween 80 and hydrogen peroxide.*
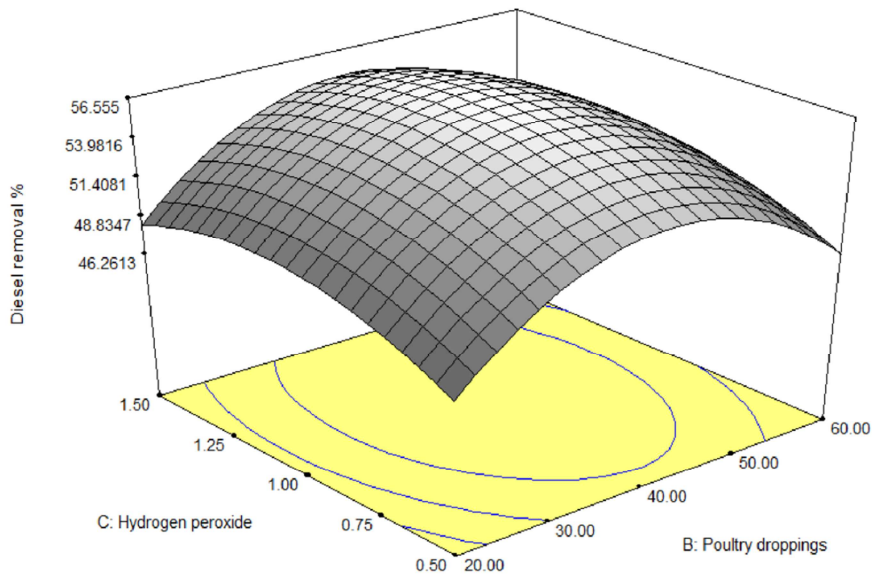

Figure 6. *Response surface 3D plots indicating interaction effects of Poultry droppings and hydrogen Peroxide.*
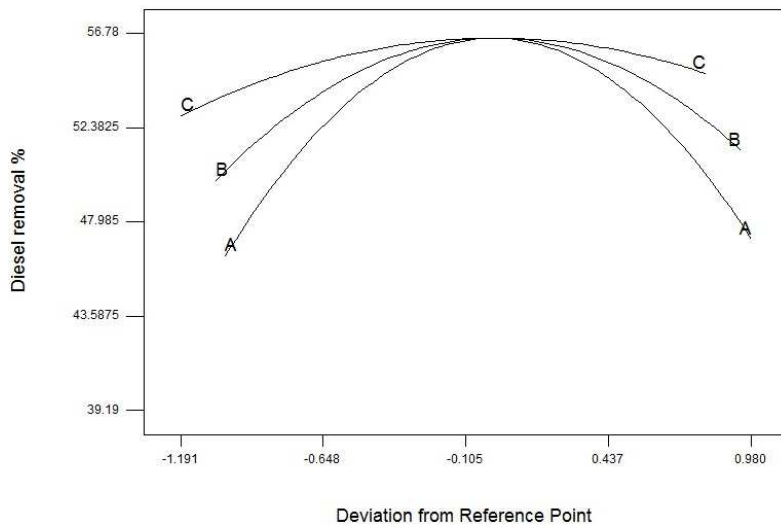

Figure 7. *Factor plot representing the individual variable effect on diesel bioremediation (A= Tween 80, B = Poultry droppings and C = Hydrogen peroxide).*

3.5. Factor Plot

The factor effect function plot was used to assess the effect of each factor graphically. From the trace plot as shown in Figure 7, it showed that each of the three variables used in the present study has its distinct effect on diesel removal by the inherent microbial populations in the soil. Gradual increase in Tween 80, Poultry droppings and hydrogen peroxide concentrations from low level (coded value –1) to a higher level (coded value +1) resulted in both increase and decrease of diesel oil degradation. Moreover, it was also observed from Figure 7 that over the range of -1 to +1 of Tween 80, the diesel degradation change in a wide range, which was also the case for Poultry droppings. However, for hydrogen peroxide the diesel oil removal did not change much over a wide range. This clearly indicates that keeping hydrogen peroxide at the optimum level, a change in Poultry droppings and Tween 80 concentrations will respectively affect the process more severely than done otherwise.

3.6. Optimization and Validation

Numerical optimization technique based on desirability function was used to determine the workable optimum conditions for the diesel oil bioremediation process. In order to provide an ideal case for biodegradation, the goal for Tween 80, Poultry droppings and hydrogen peroxide was set in range based upon the requirements of the diesel bioremediation and diesel oil removal was set on maximize. The predicted optimum (uncoded) values of Tween 80, Poultry droppings and hydrogen peroxide were found to be: 10.10ml, 41.46g and 1.10 ml, respectively, to achieve 56.565% maximum diesel oil removal; while desirability for the predicted optimum values was 0.988 (Figure 8).

Nevertheless, validation experiment was conducted to determine the optimum diesel oil removal when the biostimulation factors were set at the favorable optimum levels established above, through BBD and RSM. Standard deviation and percent error were investigated for validation of experiments. Errors between predicted and actual values were calculated according to the formula below:

$$Error = \frac{Actual - \mathrm{Pr}\,edictedError}{ActualError} X100 \qquad (5)$$

At the optimized condition for diesel removal, 56.586% diesel removal was obtained. The percentage error between the predicted and actual values was found to be 0.037%. The results clearly showed that no substantial difference was observed.
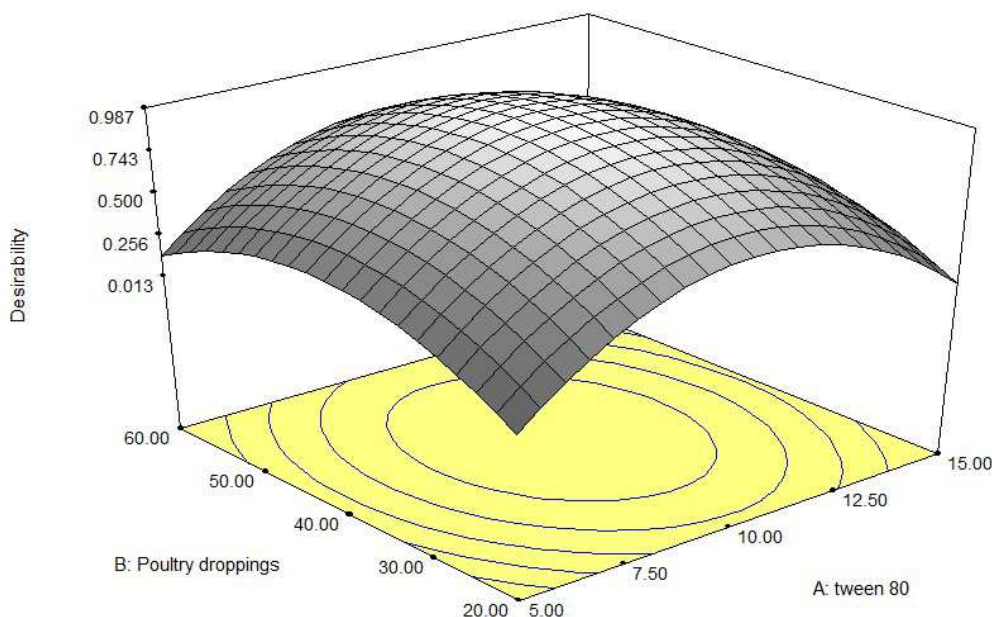

Figure 8. Desirability plot to optimize the bioremediation of diesel-polluted soil process.

4. Conclusion

This study investigated the bioremediation of diesel polluted soil and its optimization using Response Surface Methodology. The diesel contaminated soil was incubated for 28 days bioremediation period and statistical analysis was carried out. The predicted optimum parameters were Tween 80: 10.10ml, Poultry droppings: 41.46g and hydrogen peroxide: 1.10 ml. The optimal diesel oil removal was gotten to be 56.565%. At this optimum condition, the observed diesel oil removal was found to be 56.586%. It can be concluded that bioremediation resulted in petroleum hydrocarbon degradation.

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