Global Journal of Researches in Engineering: J
General Engineering
Volume 19 Issue 5 Version 1.0 Year 2019
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4596 & Print ISSN: 0975-5861

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By Baniviga, Tombari Barimme, Momoh, O.L, Yusuf & Ibiba Taiwo Horsfall
University of Port-Harcourt Choba

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GJRE-J Classification: FOR Code: 091599, 090799

PROPORTIONALOPTIMIZATIONOFCOWDUNGANDCOWBONECHARFORBIOREMEDIATIONOFCRUDEOILPOLLUTEDSOIL

Strictly as per the compliance and regulations of:

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Baniviga, Tombari Barimme [α], Momoh, O.L, Yusuf [σ] & Ibiba Taiwo Horsfall [ρ]

Abstract- In this research, attempts were made to study the effects of cow dung and cow bone char mixture for bioremediation of crude oil-polluted soil. This process of remediation was conducted ex-situ using an optimization technique termed three-level design with two factors - $\mathbf{3^2}$ design factorial. The first-order kinetics was also employed in studying the kinetics of degradation with the observed correlation of determination $(\mathbf{R^2})$ between (0.726 - 0.969).The optimization technique shows also that the optimal mix range between 20 – 38% for cow dung and 35 -50% for bone char. In terms of mass, this translates to an optimal mix of 30-57g of cow dung and 52.5 – 75gof bone char for every 2kg of soil having a 4% crude oil contamination relative to the total mass of the mixture.

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I. Introduction

Bioremediation is a treatment process that uses microorganisms (yeast, fungi, or bacteria) to degrade or break down hazardous substances into less toxic or nontoxic substances. (Walter et al, 1997) also defined soil bioremediation as the process in which most of the organic pollutants are decomposed by soil microorganisms and converted to harmless end products such as carbon dioxide $(\mathrm{CO_2})$, methane $(\mathrm{CH_4})$ and water$(\mathrm{H_2O})$. Soil microorganisms play a major role in soil bioremediation as biogeochemical agents to transform complex organic compounds into their constituent elements or into simple inorganic compounds. This process is termed mineralization. The microorganisms are adsorbed to the soil particles by the mechanism of ionic exchange. Generally, soil particles have a negative charge, and soil and bacteria can be held together by an ionic bond involving polyvalent cations (Killham, 1994).The question of the best method that should be used in oil-polluted lands depends on the biological, chemical and physical properties of both contaminants and soil. A variety of techniques had been successfully used for the cleanup of soil and groundwater contaminated with petroleum hydrocarbons, they include pump and treat of groundwater, excavation of shallow contaminated soils, and vapor extraction, etc. Efforts to remediate the negative impact of hydrocarbon pollution on the soil have resulted in several devices such as Remediation by Enhanced Natural Attenuation (RENA) which is a Land farming technique, bio-stimulation and bio-augmentation of soil biota with commercially available micro flora. RENA is a soil treatment technique commonly used, it is a full-scale bioremediation technology in which contaminated soils, sediments, and sludges are periodically tilled or turned over into the soil to aerate the waste. Soil conditions are often controlled to increase the degradation rate of the contaminant (Odu, 1978, Gradi, 1985; EPA, 1994). RENA has limitations which include the inability to properly degrade crude oil that had spilled deeply down the soil strata.

Author α σ: Department of Civil & Environmental Engineering, University of Port-Harcourt Choba, P.M.B 5323 Rivers State, Nigeria.
e-mail: yusuf.momoh@uniport.edu.ng
Author ρ: Department of Agricultural and Bioresources Engineering, Michael Okpara University of Agriculture, Abia State, Nigeria.

II. Materials and Methods

a) Sample collection

A polluted soil sample was collected using a shovel at a sample depth of 0-15cm and from three different points. The three soil samples were mixed and transported to Plant Anatomy and Physiology Research Laboratory of the University of Port-Harcourt for bioremediation. The cow dung was collected from the cowshed in the faculty of Agriculture farm site, the University of Port Harcourt and the cow bone was collected at Mgbuoba from a cow meat seller. The cow bone was taken to the University of Port Harcourt where it was crushed and burnt in a furnace. The cow dung was sun-dried for five days to drive off moisture. The soil sample was taken ex-situ from Bodo city, Gokana local government Area of Rivers State.

b) Ex-situ bioremediation procedure

The soil in the environment of the study has a previous history of crude oil contamination but in negligible concentration. 4% of crude oil (100ml) relative to the total mass of the mixture was added to each of the soil samples. The cow dung and bone char was sun-dried for the duration of 2 weeks and thereafter grounded using a mortar, pestle and a manual grinding machine. Thereafter they were sieved using a 2mm standard mesh sieve and were measured with an electronic weighing balance. The following are the design pattern for the ex-situ bioremediation procedure (1) Each sample design consists of 4% crude oil

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contamination and 2000g of soil (2) The amendments (nutrients) differ according to the proportional optimization rates. The amendments were added to the sample designs except for the control (3) the individual cells were moistened and mixed with a stirrer these stirring were conducted on an interval of four days for effective aeration.

Table 1: The design pattern for cow dung and cow bone char used as amendments in each sample

Control	2000g of soil + 100 ml of crude oil + 0g of cow dung + 0g cow bone char
S1	2000g of soil + 100 ml of crude oil + 37.5g of cow dung+ 0g cow bone char
S2	2000g of soil + 100 ml of crude oil + 75g of cow dung + 0g cow bone char
S3	2000g of soil + 100 ml of crude oil + 0g of cow dung+ 37.5g cow bone char
S4	2000g of soil+ 100 ml of crude oil+ 37.5g of cow dung + 37.5g cow bone char
S5	2000g of soil+ 100 ml of crude oil + 75g of cow dung+ 37.5g cow bone char
S6	2000g of soil+ 100 ml of crude oil + 0g of cow dung+ 75g cow bone char
S7	2000g of soil+ 100 ml of crude oil + 37.5g of cow dung+ 75g cow bone char
S8	2000g of soil+ 100 ml of crude oil + 75g of cow dung+ 75g cow bone char

c) *Optimization using the design of the experiment*

The three-level design is written as a 3^k factorial design. It means that k factors are considered and each at 3 levels. These are referred to as low, intermediate and high levels. These levels are numerically expressed as 0, 1, and 2.This is the simplest three-level design. It has two factors, each at three levels. The 9 treatment combinations for this type of design can be shown as follows:

Table 2: Optimization rates for the bio-stimulants for bioremediation of crude oil-polluted soil

Observation	Cow dung [%]	Bone char [%]	Levels
1	0	0	Low
2	25	0	
3	50	0	
4	0	25	Intermediate
5	25	25	
6	50	25	
7	0	50	High
8	25	50	
9	50	50	

The three-level designs were proposed to model possible curvature in the response function and to handle the case of nominal factors at 3 levels. The third level for a continuous factor facilitates the investigation of a quadratic relationship between the response and each of the factors.

d) *Data analysis*

Mathematical modeling was used to analyze the degradation data gotten from the experiment by applying the first-order kinetic model. Also, calculations on the bio-stimulation efficiency and percentage of degradation were conducted.

i. *Mathematical model*

$$\frac{\delta C}{\delta t} = -k_1 C \qquad [1]$$

C = concentration of degraded compound at time t,
and k_1 = first-order rate constant

$$\frac{\delta C}{\delta t} = \frac{-0.6933}{t_{1/2}} C \qquad [2]$$

$$\text{In}C_t = -k_1 t + \text{In}C_o \qquad [3]$$

$$t_{1/2} = \frac{0.6933}{k_1} = \frac{\text{In}2}{k_1} \qquad [4]$$

Where$t_{1/2}$ is the half-life
However, Eq. 3 can also be written as follows,

$$C_t = C_0 \exp(-k_1 t) \qquad [5]$$

However, the fractional efficiency of bio-degradation can be expressed as,

$$\frac{C_0 - C_t}{C_0} = F.E \qquad [6]$$

Which can be written as,

$$C_t = C_0(1 - F.E) \qquad [7]$$

Thus, substituting Eq. 7 into Eq 5 one obtains,

$$t = -\frac{1}{k_1}\text{In}(1 - F.E) \qquad [8]$$

which can be re-written as:

$$\ln\left(\frac{1}{1 - F.E}\right) = k_1 t \qquad [9]$$

The percentage degradation (%) was calculated using the formula:

$$\%\ \text{Degradation} = \frac{THC_o - THC_i}{THC_0} \qquad [10]$$

where, THC_0 = Initial THC concentration and THC_i = Residual THC concentration

ii. *Bio-stimulation efficiency*

The effectiveness of any remediation is controlled by some factors which are biotic and a biotic. It is certain that no remediation exercise can attain complete remediation, but rather the mitigation level could be high to encourage the thriving of life. The efficiency of bio-stimulation (B.E) gives insight into the treatability options offered by the various proportional optimization rates of the bio-stimulants.

$$B.E = \frac{\%THC_T - \%THC_U}{\%THC_T} \qquad [11]$$

where $\%THC_T$ = percentage removal of crude oil in the bio-stimulated or amended soil, and $\%THC_U$ = The percentage removal of crude oil in the non-bio-stimulated soil.

III. Results and Discussion

The analysis of the proportional optimization of amendments for bio-remediating the crude oil polluted soil was observed for a period of 6 weeks. The results were tabulated and illustrated in graphical patterns which shows the remediation process been carried out. These graphical representations give various insights into the optimization process.

The amendments used are cow dung and bone char. The following is the proportional optimization of the amendments.

Table 3: A table showing the optimization rates and mass of amendments of the samples

Sample	Optimization rate	Mass of biomass
1	0% Cow Dung 0% Bone Char	Cow Dung = 0g; Bone Char = 0g
2	0% Cow Dung 25% Bone Char	Cow Dung = 37.5g; Bone Char = 0g
3	0% Cow Dung 50% Bone Char	Cow Dung = 75.0g; Bone Char = 0g
4	25% Cow Dung 0% Bone Char	Cow Dung = 0g; Bone Char = 37.5g
5	25% Cow Dung 25% Bone Char	Cow Dung = 37.5g; Bone Char = 37.5g
6	25% Cow Dung 50% Bone Char	Cow Dung = 75.0g; Bone Char = 37.5g
7	50% Cow Dung 0% Bone Char	Cow Dung = 0g; Bone Char = 75.0g
8	50% Cow Dung 25% Bone Char	Cow Dung = 37.5g; Bone Char = 75.0g
9	50% Cow Dung 50% Bone Char	Cow Dung = 75.0g; Bone Char =75.0g

a) *Physiochemical analysis of cow dung and bone char*

The physicochemical analysis was gotten from the amendments stating the carbon %, ash content %, volatile matter %, Nitrogen %, and Phosphorus contents as shown below:

Table 4: Result showing the Physiochemical Analysis of Cow dung and Bone Char

Sample Identity	% Carbon	% Ash content	Volatile Matter	Phosphorus (mg/kg)	% Nitrogen	C/P ratio	C/N ratio
Cow Dung	2.91	79.4	20.6	1.304	0.045	2.231595	64.66667
Bone Char	2.4	60.7	39.3	1.712	0.007	1.401869	342.8571

Table 5: Analysis of THC

Sample Identity	Week 1 THC (Mg/Kg)	Week 2 THC (Mg/Kg)	Week 3 THC (Mg/Kg)	Week 4 THC (Mg/Kg)	Week 5 THC (Mg/Kg)	Week 6 THC (Mg/Kg)
Control 0%CD 0% BC	7500	5850	5750	4450	4000	3400
0%CD 25% BC	7350	4500	3750	3300	2850	2250
0%CD 50% BC	4500	3000	2850	2700	2250	1800
25% CD 0% BC	7200	3600	3300	3000	2700	1650
25%CD 25% BC	4500	3900	3150	2400	2100	1200
25%CD 50% BC	4500	4350	3000	2550	2250	1350
50%CD 0% BC	4650	3300	2700	2250	1800	1200
50%CD 25% BC	6000	3750	2850	2325	1875	1650
50%CD 50% BC	7500	3300	2700	2400	1950	1650

CD= Cow Dung
BC= Bone Char

b) *Statistical analysis of THC*

The biodegradation of THC in the soil of 4% crude oil contamination was analyzed for the various optimization rates using the statistical t-test by the following conditional statements below: If P> 0.05 we should accept the null hypothesis (H_o); of no significant effect on remediation process and reject the alternative hypothesis (H_i); of a significant effect on remediation.

If P<0.05 we reject the null hypothesis(H_o); of no significant effect on the remediation process and we accept the alternative hypothesis (H_i); of a significant effect on the remediation process. From the data gathered, the table shows the various P-value and remarks of the sample.

Table 7: Summary of t-test table for THC degradation

Samples	Rate	Mass	P-Value	Remark
1	Control	CD=0g; BC=0g	-	-
2	0%CD 25% BC	CD=37.5g;BC=0g	4.97 E-03	Significant Difference

3	0%CD 50% BC	CD=75.0g; BC=0g	3.84E-04	Significant Difference
4	25% CD 0% BC	CD=0g; BC=37.5g	3.99E-03	Significant Difference
5	25%CD 25% BC	CD=37.5g; BC=37.5g	4.90E-05	Significant Difference
6	25%CD 50% BC	CD=75.0g; BC=37.5g	2.87E-04	Significant Difference
7	50%CD 0% BC	CD=0g; BC=75.0g	1.51E-05	Significant Difference
8	50%CD 25% BC	CD=37.5g; BC=75.0g	1.20E-04	Significant Difference
9	50%CD 50% BC	CD=75.0g; BC=75.0g	6.46E-03	Significant Difference

CD = Cow dung; BC = Bone Char

Further analysis of the various proportional optimization rates for bioremediation was also ascertained using the student's t-test. From the student's t-test results shown, a significant level ($P<0.05$) was observed for treatment using the various optimization rates of amendments in Samples 2, 3, 4, 5, 6, 7, 8, 9 as compared with control (Sample 1). Thus, there was a significant difference in all the samples. So, we accept the alternative hypothesis (H_i); which indicates a significant reduction in crude oil contamination for the various optimization rates of amendments in the samples.

c) Percentage of Degradation

The percentage degradation for the various sample blocks is shown in the bar chart below. From the chart, it can be deduced that sample 9 has the greatest degradation of Total hydrocarbon Content (THC). Thus, in all samples the percentage of degradation was above 50%, showing effective bioremediation.

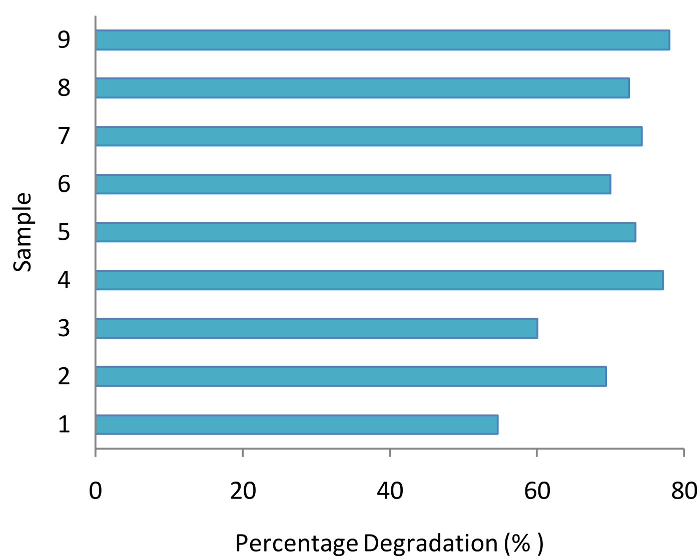


Figure1: Bar Chart Showing the Percentage Degradation of the Nine (9) Samples

$$\%degradation=75.30540+0.34274\times CD+6.77319\times BC-2.74977\times CD^2-5.34756\times BC^2-0.38172\times CD\times BC$$

where BC = Bone char rate [%]; CD = Cow dung rate [%]

d) Optimal mix of cow dung and bone char using response surface methodology (RSM)

Response surface methodology (RSM) is a collection of statistical and mathematical techniques for building an empirical model. The objective is to optimize a response (output variable) which is influenced by several independent variables (input variables i.e. cow dung and bone char rates).

Using the response surface methodology (RMS), the optimal THC degradation Efficiency is between 76.50 – 80.00%. The optimal mix of the cow dung is between 20 – 38% and the bone char is 35 - 50%.

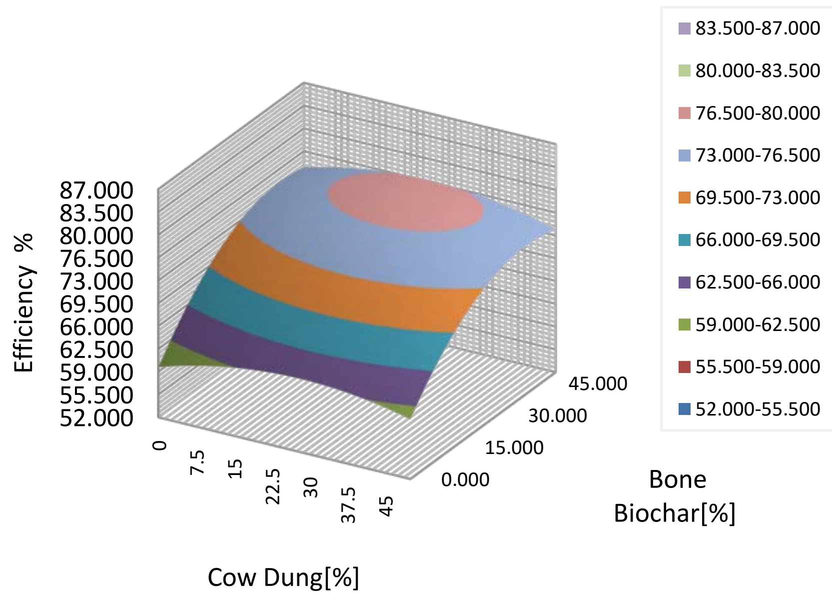


Figure 2: Contour plot (3D view) showing the THC degradation efficiency of the Bio-Stimulants in the amended soil

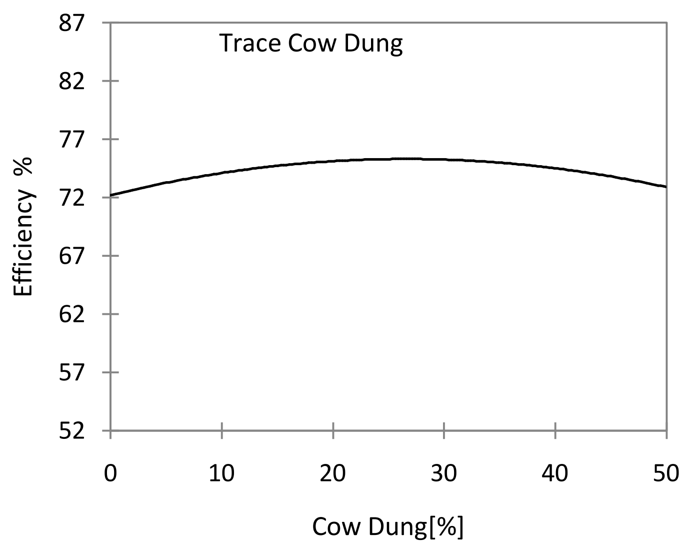


Figure 3: A plot of Efficiency at various optimization rates of Cow Dung

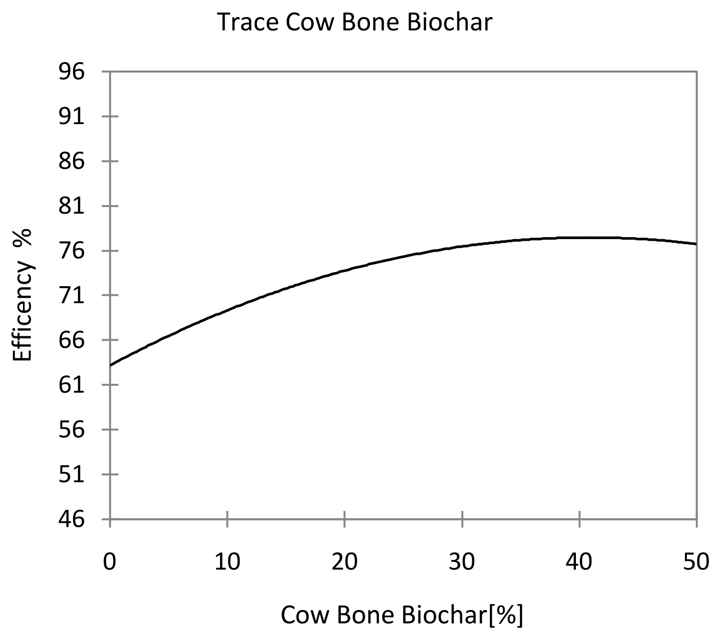


Figure 4: A plot of Efficiency at various optimization rates of Bio char

Table 8: Experimental design table for various samples observation

Sort Order	Run Order	Repetition	Cow Dung	Cow Bone Biochar	% Degradation
1	1	1	0	0	54.67
2	2	1	25	0	69.39
3	3	1	50	0	60
4	4	1	0	25	77.08
5	5	1	25	25	73.33
6	6	1	50	25	70
7	7	1	0	50	74.19
8	8	1	25	50	72.5
9	9	1	50	50	78

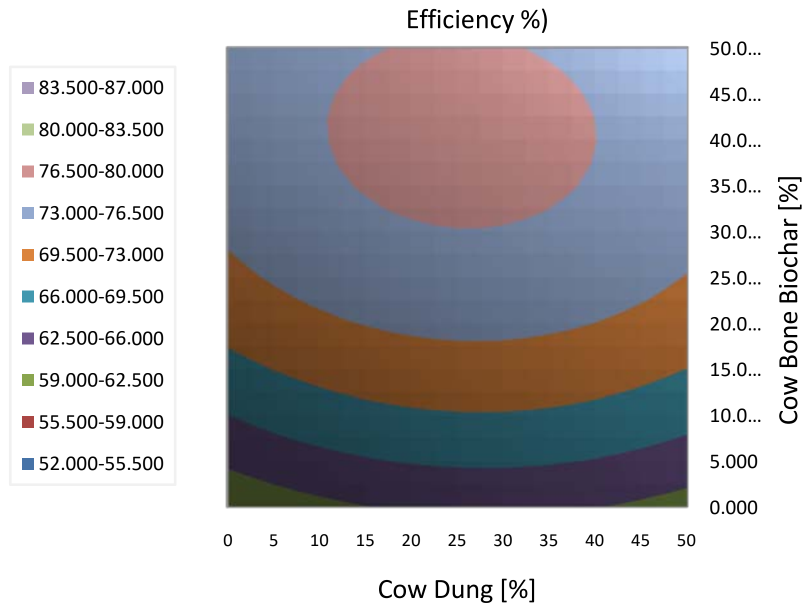
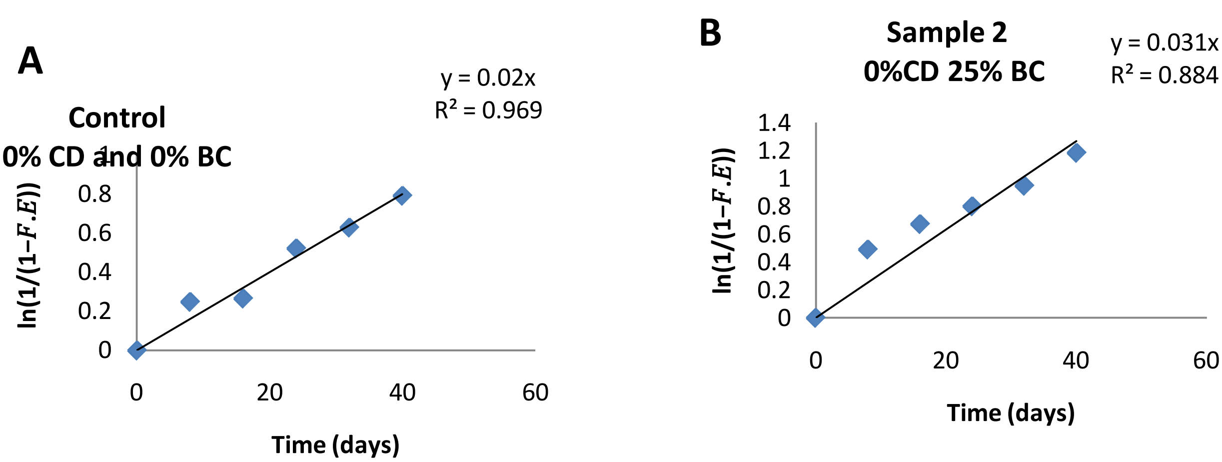
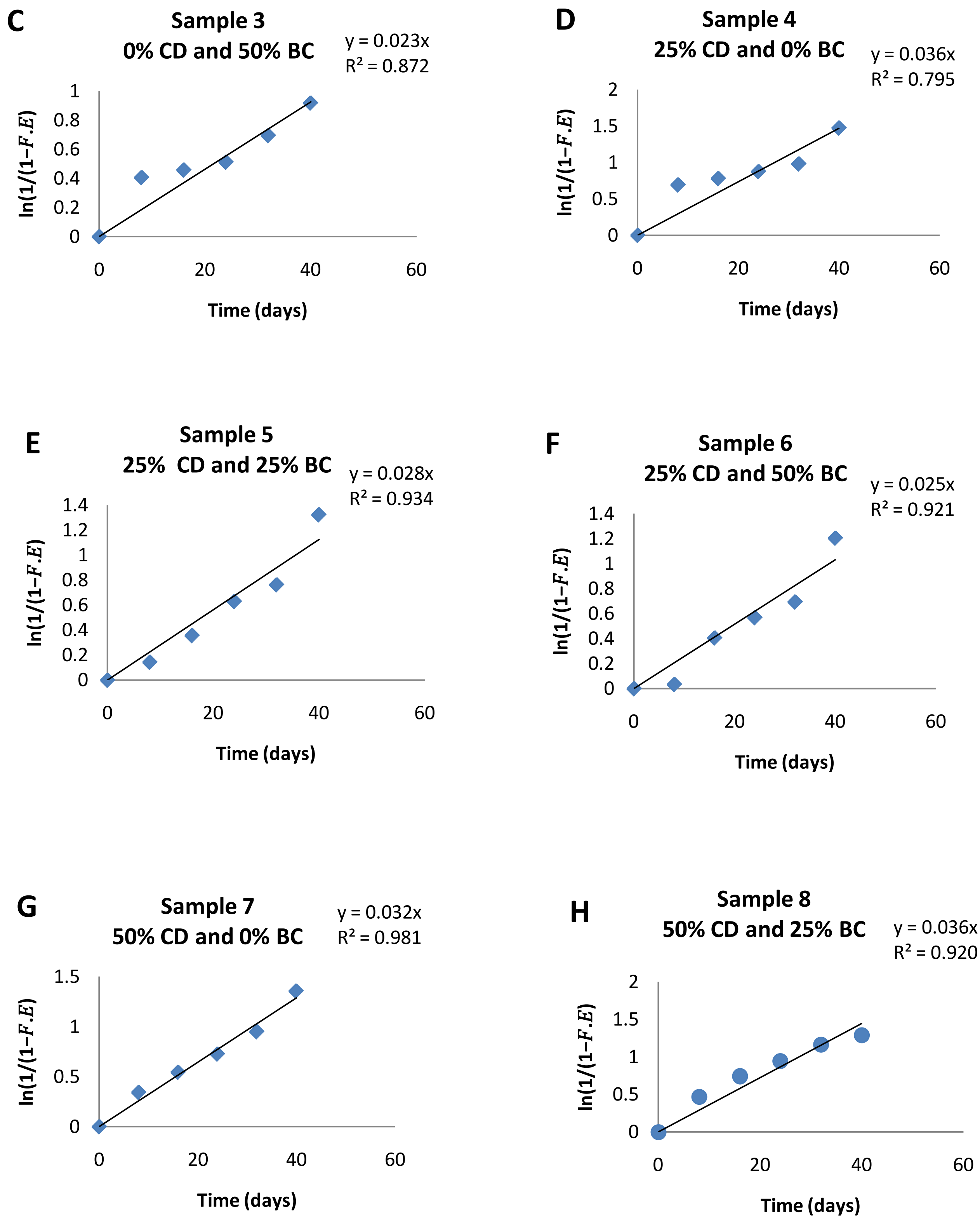


Figure 5: A Contour plot showing the Efficiency from the various optimization rates of the Bio-Stimulants.

e) Kinetics of total hydrocarbon (THC) degradation

The kinetics of THC degradation was studied using first-order kinetics, which proposes that the rate of change of substrate is directly proportional to the concentration of the substrate. High K value implies high degradation rate. The fractional efficiency was plotted against time for each of the samples.




C
Sample 3
0% CD and 50% BC
y = 0.023x
R² = 0.872
ln(1/(1−F.E)
Time (days)
D
Sample 4
25% CD and 0% BC
y = 0.036x
R² = 0.795
ln(1/(1−F.E)
Time (days)
E
Sample 5
25% CD and 25% BC
y = 0.028x
R² = 0.934
ln(1/(1−F.E)
Time (days)
F
Sample 6
25% CD and 50% BC
y = 0.025x
R² = 0.921
ln(1/(1−F.E)
Time (days)
G
Sample 7
50% CD and 0% BC
y = 0.032x
R² = 0.981
ln(1/(1−F.E)
Time (days)
H
Sample 8
50% CD and 25% BC
y = 0.036x
R² = 0.920
ln(1/(1−F.E)
Time (days)

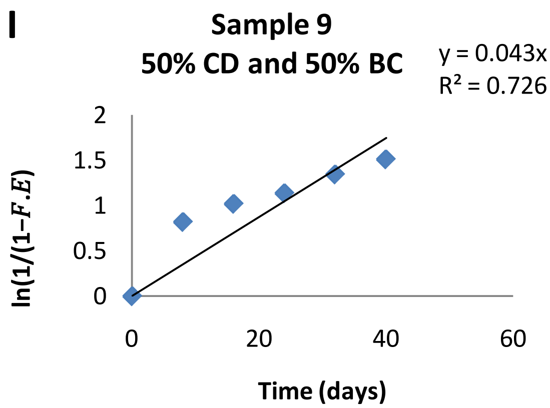


Figure 6: (A) First Order Kinetic pattern of THC reduction for Control (B) Sample 2 (C) Sample 3 (D) Sample 4 (E) Sample 5 (F) Sample 6 (G) Sample 7 (H) Sample 8 (I) Sample 9

From the above graphs in figure 6, it was observed that the coefficient of determination(R^2) indicates a positive correlation for the reduction in Total Hydrocarbon Content (THC) with respect to time with a biodegradation rate constant of $0.02\mathrm{Day}^{-1}$, $0.031\mathrm{Day}^{-1}$, $0.023\mathrm{Day}^{-1}$, $0.036\mathrm{Day}^{-1}$, $0.028\mathrm{Day}^{-1}$, $0.025\mathrm{Day}^{-1}$, $0.032\mathrm{Day}^{-1}$, $0.036\mathrm{Day}^{-1}$ and $0.043\mathrm{Day}^{-1}$ for the Samples 1-9.The biodegradation rate constant differs for each of the samples, it can be noted that the higher the rate constant the smaller the half-life in the sample. Sample 9 has the highest biodegradation rate constant of $0.043\mathrm{Day}^{-1}$ and a half-life of 16 days followed by sample 8 with a rate constant of $0.036\mathrm{Day}^{-1}$ and a half-life of 19 days, Followed by sample 4 with a rate constant of $0.036\mathrm{Day}^{-1}$ and a half-life of 19 days, followed by sample 7 with a rate constant of $0.032\mathrm{Day}^{-1}$ and a half-life of 22 days, followed by sample 2 with a rate constant of $0.031\mathrm{Day}^{-1}$ and a half-life of 22 days, followed by sample 5 with a rate constant of $0.028\mathrm{Day}^{-1}$ and a half-life of 25 days, followed by sample 6 with a rate constant of $0.025\mathrm{Day}^{-1}$ and a half-life of 28 days, followed by sample 3 with a rate constant of $0.023\mathrm{Day}^{-1}$ and a half-life of 30 days, followed by control with a rate constant of $0.02\mathrm{Day}^{-1}$ and a half life of 35 days. The remediation rate in descending order for hydrocarbon degradation is given as: Sample 9> sample 8 >sample 4> sample7 >sample 2 > sample 5 > sample 6> sample 3> control.

Table 9: First order decay equation, biodegradation rate constant, half-life

Samples	Rate	Mass	Kinetics Equation	K (day^{-1})	Half-Life $t^{\frac{1}{2}}$(days)	R^2	% D	B.E (%)
1	Control	CD=0g; BC=0g	y = 0.02x	0.02	35	0.969	54.67	
2	0%CD 25%CBC	CD=37.5g; BC=0g	y = 0.031x	0.031	22	0.884	69.39	21.21
3	0%CD 50%CBC	CD=75.0g; BC=0g	y = 0.023x	0.023	30	0.872	60	8.89
4	25% CD 0%CBC	CD=0g; BC=37.5g	y = 0.036x	0.036	19	0.795	77.08	29.08
5	25%CD 25%CBC	CD=37.5g; BC=37.5g	y = 0.028x	0.028	25	0.934	73.33	25.45
6	25%CD 50%CBC	CD=75.0g; BC=37.5g	y = 0.025x	0.025	28	0.921	70	21.90
7	50%CD 0%CBC	CD=0g; BC=75.0g	y = 0.032x	0.032	22	0.981	74.19	26.32
8	50%CD 25%CBC	CD=37.5g; BC=75.0g	y = 0.036x	0.036	19	0.92	72.5	24.60
9	50%CD 50%CBC	CD=75.0g; BC=75.0g	y = 0.043x	0.043	16	0.726	78	29.91

CD = Cow dung; BC = Bone Char; % D = Percentage degradation; B.E = Bio-stimulation efficiency; R^2= Correlation coefficient.

IV. Conclusion

The percentage degradation for the various sample blocks shows that sample 9 has the greatest degradation of Total hydrocarbon Content (THC). Also, in all samples the percentage of degradation was above 50%, showing effective bioremediation.Using the response surface methodology (RSM), the optimal THC degradation Efficiency is between 76.50 – 80.00%.The optimal mix proportion for cow dung is between 20 – 38% and for bone char, it is between 35 -50%. In terms of mass, the proportional optimal mix for the cow dung is between 30 – 57g and bone char is 52.5 – 75g for every 2kg of soil with a 4% crude oil contamination relative to the total mass of the mixture.

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