

Kinetic Modelling and Half-Life Study of the Bioremediation of Used Motor Oil Contaminated Soil using Animal Dung as Stimulants

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ABSTRACT: This work was conducted to compare the bioremediation potential of horse dung, elephant dung, donkey dung and their combination in microcosms labelled M_2 , M_3 , M_4 , and M_5 respectively on the bioremediation of used motor oil contaminated soil. The bioremediation studies were investigated for 42 days in the laboratory. The results of the studies showed that there was a positive relationship between the rate of reduction of used motor oil (UMO) and the presence of a horse, elephant and donkey dung in all the microcosms. The biodegradation data of the oil fitted well to the first and second order kinetic models. The models showed that UMO contaminated soil microcosms amended with horse dung (M_2), elephant dung (M_3), donkey dung (M_4), and their combination (M_5) had lower half-life times as well as higher biodegradation rate constants when compared with the unamended control soil (M_1). The ANOVA statistical analysis results showed that the addition of the animal dung significantly influenced the biodegradation of the UMO in the polluted soil at 95% confidence level since the probability value was estimated to be less than 0.05. Also, the Tukey's HSD test ($p=0.05$) showed that the Biodegradation of UMO in the microcosms was significantly influenced by horse, elephant, donkey dung, and their combination as amendment agents. The results of the bioremediation efficiency and the kinetic modelling, showed that the bioremediation potential of the animal dung as stimulants is in the order $M_3 > M_2 > M_4 > M_5$.

KEYWORDS -Biodegradation, bioremediation, donkey dung, elephant dung, horse dung, kinetics, microcosm, used motor oil.

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I. INTRODUCTION

Since when the exploration of oil began, the environment (soil and water) have been highly polluted by oil and its derivatives [1]. The pollution of the environment is caused by the increase in the oil exploration and population, which led to the demand for the crude oil and its derivatives. In today's world, oil spills at auto-mechanic workshops have been left uncared for over the years in many countries, and continuous accumulation of the oil is of high environmental concern as a result of hazard associated with it [2].

Dispersion, dilution, sorption, volatilisation, abiotic transformations, excavation, and containment in secured landfills, vapour extraction, incineration, are essential technologies for the remediation of oil-contaminated soil [3]. However, their usage is limited because they are expensive to be applied at large scale, toxic to the environment, involved sophisticated technology, destroy soil texture and its characteristics and do not always result in the complete neutralisation of the pollutants [2].

The naturally occurring process by which microorganisms convert environmental pollutants into the harmless by-products is termed bioremediation [2]. Since activities of microbes influence the biodegradation of oil, there is a need for the adjustment of the nutrients (nitrogen and phosphorus) to enhance the microbial proliferation of the indigenous microorganisms present in the polluted environment. To stimulate the microbial proliferation in the contaminated soil in order to enhance the biodegradation process, the soil is amended with organic stimulants. [4],[5],[6].

The objectives of this work are to compare the bioremediation potentials of animal dung (horse dung, elephant dung, donkey dung and their combination) as stimulants, determine the bioremediation efficiency, and the bioremediation kinetic parameters for the treatment in all the microcosms for first and second order kinetic models.

II. MATERIALS AND METHODS

2.1 Sample Collection

Surface soil contaminated naturally with used motor oil (0 – 10 cm) was collected from old Dan Gombe Auto-Mechanics Workshop situated along Jos Road in Bauchi, Bauchi State – Nigeria in a black polythene bag and transported to Abubakar Tafawa Balewa University Bauchi Chemical Engineering Reaction Laboratory. The soil awaiting microbial analysis was stored at 4°C in a refrigerator. The elephant dung was collected from Yankari Games Reserve Bauchi State, Nigeria, the donkey dung was obtained from Gwalameji village opposite Federal Polytechnic Bauchi and the horse manure was sourced from horse stable in Kobi Street Bauchi, Bauchi State, Nigeria

2.2 Preliminary Analysis of the Soil Sample

The contaminated soil sample was subjected to the following physicochemical and microbial analysis. The pH was determined according to [2], moisture content was determined according to [7], the organic carbon was determined according to [8]. The particle density was determined according to [9], bulk density was determined according to [10], and their values were used for calculating the soil porosity [2]. The available phosphorus in three samples was determined using the spectrophotometer while total nitrogen was obtained by the Kjeldahl method [2]. The pure bacterial isolate was characterized and identified using the standard procedure based on Bergey's manual [11]. The total heterotrophic bacterial count was determined by inoculating 0.1 ml of the serially diluted sample on the nutrient agar (oxid) plate using the spread plate method [12], the oil grease content was determined using Soxhlet extraction method [13]. The physicochemical and microbiological analyses of the soil and different animal manures were done in duplicate.

2.3 Experimental Design and Treatment

One thousand five hundred grams (1500 g) of sieved (2mm) soil was mixed with 10% w/w [14],[15],[16],[17] of different animal dungs (horse dung, elephant dung, donkey dung and the combination of the three) in plastic containers (microcosms). Control vessel consisting of contaminated soil without amendment was set up. The moisture content was adjusted and maintained at 20% water holding capacity [18] by the addition of distilled water. It was kept at room temperature (28 ± 2°C) and the content of each microcosm was pulverised two times in a week for aeration. Periodic sampling from each of the microcosms was done weekly for six weeks to determine the residual oil and grease content and microbial count. The design of the experimental setup is as shown in Table 1.

Table 1: Summary of the Experimental Design

Microcosms	Treatment
M ₁	1500 g Polluted soil
M ₂	1500 g polluted + horse dung
M ₃	1500 g polluted + 10% elephant
M ₄	1500 g polluted + 10% donkey dung
M ₅	1 500 g polluted + 10% combination of the three dung

2.4 Determination of Oil and Grease Content

Soxhlet extraction method was used for the quantifying the oil and grease [13]. Sodium sulphate was purified by drying overnight in an oven at 105°C. Round Soxhlet flask was dried at 105°C for 30 min. After cooling, the weight of the round Soxhlet flask and boiling chip was recorded (W₂). 3.0 g of contaminated soil was mixed with 3.0 g of anhydrous Na₂SO₄ and placed in a cellulose extraction thimble. 60 ml of n-hexane was added to the flask, and the oil was extracted for 1 hours. The residual oil was determined by evaporating the n-hexane in a hot water bath; the round bottom flask was allowed to cool and weighed again (W₁). Residual oil/grease content in the soil was calculated using (1) and (2).

$$\text{Oil and grease content (ppm)} = \frac{\text{The gain in weight of the flask}}{\text{Weight of soil}} \times 1000 \quad (1)$$

$$\text{Oil and grease content (ppm)} = \frac{W_1 - W_2}{W} \times 1000 \quad (2)$$

W₁ = weight of flask, boiling chips and residue after evaporation of hexane (mg)

W₂ = weight of round flask and boiling chips (mg)

W = the weight of the contaminated soil (g)

The percentage degradation (D) of the oil was determined using (3)

$$D = \frac{R_{O\&G_i} - R_{O\&G_r}}{R_{O\&G_i}} \times 100 \quad (3)$$

Where $R_{O\&Gr}$ and $R_{O\&Gi}$ are the residual and initial oil and grease concentrations, respectively.

2.5 Determination of Total Heterotrophic Bacterial Count

The enumeration of the total heterotrophic bacterial count present in the microcosms was determined by spread plate techniques. The samples were subjected to serial dilution which was plated on nutrient agar (NA) oxoid and incubated at $(28\pm 2^{\circ}\text{C})$ for 24 h and plate that yielded count between 30 – 300 colonies were counted [14].

III. RESULTS AND DISCUSSIONS

3.1 Physicochemical Properties of Soil and Organic Wastes

Table 2 shows the physical and chemical properties of the animal dung and the used motor oil contaminated soil used for the study. The high level of percentage total organic carbon (24.74 %) in the polluted soil was as a result of the used motor oil in the soil whose oil and grease content (112 734 mg/kg) was above the safe limit of 500 mg/kg set by the Nigeria Ministry of Environment [19], hence the need for the remediation of the polluted soil. The soil pH (6.9) was within the acceptable limit of 5.5 – 8.5 for effective bioremediation according to [20]. The soil moisture content (2.57 %) fell out of the limit of 12 – 25 % required for optimum growth and proliferation of microbes [21] hence the need for the moisture content adjustment.

The nitrogen content (0.42%) of the polluted soil was low, hence the need for the amendment with organic wastes (donkey, elephant, and horse dung). The nitrogen content of the donkey dung, elephant, and horse dung was found to be 0.67%, 1.96% and 0.98% respectively which is one of the limiting nutrient required for effective bioremediation.

Table 2: Physicochemical properties of the sample

Parameter	Samples			
	Soil	Horse	Elephant	Donkey
pH	6.9	6.8	6.9	5.8
Nitrogen (%)	0.42	0.98	1.96	0.67
Organic C (%)	24.74	10.51	4.79	6.92
Phosphorus (%)	0.67	0.46	0.21	0.16
Moisture (%)	2.57	ND	ND	ND
Oil & Grease (ppm)	112734	ND	ND	ND

3.2 The heterotrophic bacterial count in the contaminated soil

Table 3 gives the total heterotrophic bacteria count (THBC). From the Table, THBC in the used motor oil-contaminated soil was found to be $9.10\text{E}+08$. The density of the indigenous bacteria in the contaminated soil was enough for effective bioremediation since it exceeded the minimal value of $1.00\text{E}+05$ required.

Table 3: Heterotrophic Bacteria Count in the Contaminated Soil

Sample	Total Heterotrophic Bacteria Count (CFU g^{-1} soil)
Contaminated Soil	9.10×10^8

3.3 Oil and Grease (O&G) for the Microcosms

According to [2], the oil and grease content is a better bioremediation index for studying the extent of degradation of pollutant in used motor oil contamination since the concentration of total petroleum hydrocarbon is low due to the decrease in C-H bond in used motor oil. Fig 1 shows the residual oil and grease in the microcosm with bioremediation time. As shown in the Figure, the O&G percentage degradation was observed to increase with the bioremediation time, which is a normal trend for an exemplary oil biodegradation process.

From the results (Fig.1), it was noticed that the reduction of oil and grease of the contaminated soil was relatively fast for the first 14 days of the biodegradation process in the amended microcosms M_2 , M_3 , M_4 , and M_5 when compared to that of the unamended microcosms M_1 . After the first 14 days, there was reduction of O&G in microcosms M_1 , M_2 , M_3 , M_4 and M_5 from 112 734, 103 149, 104 642, 104 469, and 104285 mg kg^{-1} to 89 450, 42 427, 41 756, 52 596, and 59 536 mg kg^{-1} respectively which corresponded to 29.5, 66.9, 71.4, 60.8, and 52.2 % reduction in O&G contents.

After 42 days of the remediation process, the concentration of the used motor oil reduced to 63 655, 21 265, 17 025, 31 997 and 34 131 mg kg^{-1} and correspondingly 43.5, 79.4, 83.74, 69.4 and 67.3% O&G reduction for microcosms M_1 , M_2 , M_3 , M_4 and M_5 respectively. It was noticed that the degradation of used motor oil in M_2 , M_3 , M_4 and M_5 resulted in effective bioremediation response with M_3 having the highest bioremediation response (83.7 % loss in O&G contents).

3.4 Microbial analysis

The results of total heterotrophic bacteria count (THBC) throughout the remediation process are presented in Fig. 2. It was observed that microbial growth profile followed a common microorganisms' growth

pattern with lag, exponential, stationary, and death phases. All the microcosms showed a similar trend of lag phase which is the period of adaptation to the new environment. This period remained for seven (7) days. Between days 7 and 21, the microcosms followed a similar pattern of the exponential phase, the period of maximum oil biodegradation. After 42 days of incubation, the THBC in microcosms M₁, M₂, M₃, M₄, and M₅ were found to be 2.36E+09, 3.35E+09, 3.88E+09, 4.26E+09 and 3.57E+09 respectively. The trend observed in the microbial growth corresponded with the percentage degradation of the oil and grease. Hence, it is clear that the bacteria utilised the used motor oil as their carbon and energy source.

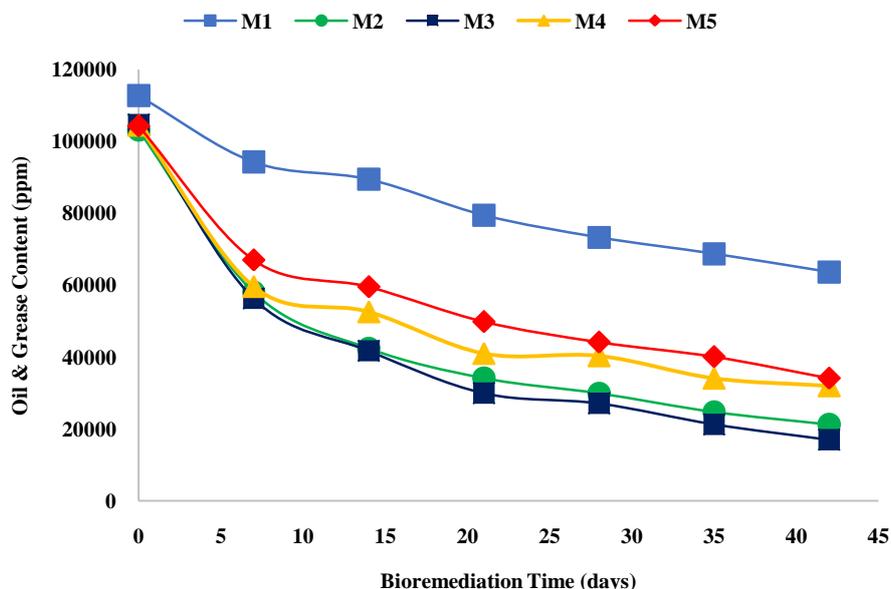


Figure 1: Variation of oil and grease (mg kg⁻¹) with bioremediation time (days).

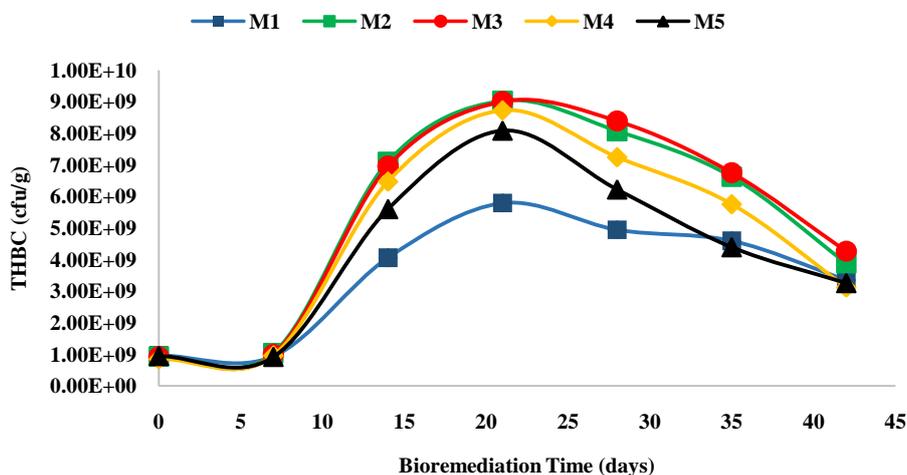


Figure 2: Variation of total heterotrophic bacteria count (THBC) with bioremediation time.

3.5 Bioremediation Kinetic models fitting

To investigate the biodegradation process of the used motor oil present in the microcosms, first order, and second kinetic models [3],[22],[23],[24],[21],[25],[26] were used to estimate the biodegradation rate constant and half-life to compare the effectiveness of the stimulants in enhancing the degradation of used motor oil in the microcosms. (4) and (5) gives the first and second order kinetic model expressions while (6) and (7) gives the expressions for calculating the half-life (t_{1/2}) in days, of the biodegradation process.

$$\ln R_{O\&Gr} = -k_1 t + \ln R_{O\&G0} \tag{4}$$

$$\frac{1}{R_{O\&Gr}} = k_2 t + \frac{1}{R_{O\&G0}} \tag{5}$$

$$t_{1/2} = \frac{\ln 2}{k_1} = \frac{0.693}{k_1} \tag{6}$$

$$t_{1/2} = \frac{1}{k_2 R_{TPH0}} \tag{7}$$

Where $R_{O\&Gr}$ and $R_{O\&G0}$ are the residual and initial concentration of the oil and grease (mg/kg) respectively, t is the bioremediation time (day) while k_1 (day^{-1}) and k_2 ($\text{kgmg}^{-1}\text{day}^{-1}$) are respectively the first and second order biodegradation constants.

Table 4 gives the parameters obtained from the two kinetic model equations. The linear regression coefficient of determination R^2 was used to evaluate the model that best fit the experimental data. The R^2 values gotten from the plots of all the studied microcosms ranges from 0.8718 to 0.9907 for the first order and 0.9485 to 0.9958 for second-order kinetic models. The model with relatively high R^2 value best described the degradation of hydrocarbon in the microcosms.

Results from Table 4 showed that the biodegradation of used oil in soil amended with elephant dung (M_4) had a higher rate constant and lower half-life for the first order ($k_1 = 0.0399 \text{ d}^{-1}$ and $t_{1/2} = 17.37$ days) and second order ($k_2 = 1\text{E-}06 \text{ kgmg}^{-1}\text{d}^{-1}$ when compared with the soil amended with the other dung.

3.6 Analysis of Data

One-way analysis of variation (ANOVA) at 5% probability level was used to analyse the data. The means of the amendment in all the microcosms were tested for the level of significant differences at 5% probability using Turkey's Honestly Significant Difference (HSD) test. The analysis was done using the statistical package for social science, version 16.0 (SPSS Inc., Chicago, IL, USA). Hypothesis: Is there a significant difference in the mean removal of oil and grease (O&G) among the stimulants (animal dung) at 0.05 significance level? Decision: Reject the null hypothesis if the p-value calculated is less than the p – significance level (0.05). Table 5 shows the one-way ANOVA conducted to compare the bioremediation potential of the horse dung, elephant dung, donkey dung, and their combination (stimulants)

The results showed that the percentage oil and grease removal means are not equal for all the microcosms (M_1, M_2, M_3, M_4, M_5) since the calculated p-value (2.24E-15) is less than the alpha significance level (0.05), so the null hypothesis is rejected. In order to determine which treatment group differs or how many treatment groups differ, the Turkey's HSD test at 5% significance level was carried out to know the significant difference in the bioremediation potential between the horse dung (M_2), elephant dung (M_3), donkey dung (M_4), and their combination (M_5).

Results of Turkey's HSD test (Table 6) showed that there were significant differences between the four amendment agents and the control as well as between the amendment agents.

3.7 Bioremediation Efficiency

The effectiveness of the biostimulants was compared by evaluating the bioremediation efficiency using (7).

$$\% \text{ Efficiency}(Eff) = \frac{\% R_{(a)} - \% R_{(u)}}{\% R_{(a)}} \times 100 \tag{7}$$

Where: $\% R_{(a)}$ is the percentage degradation of oil and grease content in the amended soil, $\% R_{(u)}$ is the percentage degradation of oil and grease content in the unamended soil. The results of the Eff (%) are presented in Table 5. Based on the result, it was found that soil amended with elephant dung (M_3) is more effective than soil amended with horse dung (M_2), donkey dung (M_4) and their combination (M_5).

Table 4: Summary of the Samples Rate Constants, Half-lives and Correlation Coefficients for First and Second Order

sample	First Order			Second Order		
	k_1 (d^{-1})	$t_{1/2}$ (d)	R^2	k_2 ($\text{kg mg}^{-1}\text{d}^{-1}$)	$t_{1/2}$ (d)	R^2
M1	0.0130	53.3	0.9763	2E-07	47.1	0.9920
M2	0.0346	20.0	0.9287	9E-07	9.9	0.9958
M3	0.0399	17.4	0.9463	1E-06	9.7	0.9863
M4	0.0252	27.5	0.8718	5E-07	19.7	0.9573
M6	0.0239	29.0	0.9358	4E-07	22.0	0.9856

Table 5: Analysis of variance (ANOVA) for the percentage oil and grease degradation in the microcosms

	Sum of Squares	Degree of Freedom	Mean Square	F-value	p-value
Between Groups	2927.253	4	731.813	3.042E+03	2.24E-15

Within Groups	2.405	10	0.241
Total	2929.659	14	

Table 6: Turkey's HSD Test for the Oil and Grease Percentage Degradation in the Microcosms.

Treatments	Mean O&G degradation (%)	Standard error	Remarks
M ₁ (Control)	43.54 ^a	0.2771	Significant difference
M ₂ (Horse dung)	79.38 ^b	0.4532	Significant difference
M ₃ (Elephant dung)	83.74 ^c	0.3407	Significant difference
M ₄ (Donkey dung)	69.37 ^d	0.0318	Significant difference
M ₅ (Their combination)	67.27 ^e	0.0404	Significant difference

Means that do not have the same letter are significantly different

Table 7: Percentage Degradation of Used Oil and Bioremediation Efficiency of the Stimulants after 42 Days of Bioremediation

Treatments	O&G degradation (%)	Eff (%)
M ₁ (Control)	43.54	
M ₂ (Horse dung)	79.38	45.16
M ₃ (Elephant dung)	83.73	48.01
M ₄ (Donkey dung)	69.37	37.24
M ₅ (Their combination)	67.27	35.28

IV. CONCLUSION

In this work, the biostimulation potentials of horse dung, elephant dung, donkey dung and their combination were investigated in five microcosms to remediate used motor oil contaminated soil. The residual used motor oil reduction as well as the microbial data after 42 days of incubation, revealed the occurrence of biodegradation of used motor oil and increased in the population of the indigenous heterotrophic bacteria count in all the microcosms. Based on the results obtained, it was observed that the application of horse dung (M₂), elephant dung (M₃), donkey dung (M₄) and their combination (M₅) to stimulate the indigenous microbes in the contaminated soil gave percentage O&G degradation of 79.38 %, 83.73 %, 69.37 %, and 67.27 % respectively with elephant dung being more effective than the horse dung, donkey dung and the combination of the three dung.

The rate constants and half-lives of the studied models (first and second order kinetics) calculated showed that the soil amended with elephant dung had the lowest half-life value for both first order (9.7 days) and second order (17.4 days) as well as the highest rate constant value for both first (0.0399 day⁻¹) and second order (1E-06 kgmg⁻¹d⁻¹) respectively. Therefore, elephant dung was more effective than the horse dung, donkey dung, and their combination as biostimulant.

The results of the analysis of variation (ANOVA) and Turkey's HSD test showed that there was a significant difference in the bioremediation potential of the horse dung, elephant dung, donkey dung, and their combination. Also, results of the bioremediation efficiency revealed that elephant dung is the most effective biostimulants.

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