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Kinetic Modelling of bioremediation of diesel in clay polluted soil

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Abstract
The paper modelled and appraised the effect of contaminant concentration on the bioremediation of clay soil artificially polluted by diesel. This is achieved by preparing microcosms containing 50 g of clay soil, 4% w/w NPK fertilizer, deionized water and diesel in the range of 3-8% w/w. Samples were drawn and extracted for GC analysis and microbial viable count after every week. The results showed that there is low degradation in all samples. The degradation rate constants were low (0.0082 to 0.0243/day) leading to moderate half-life times (28.5-84.5 days). The kinetics model used was fitted to the experimental data and it has been found that highest degradation rate constant (0.0243/day) and the lowest half-life time were obtained at 5% w/w diesel concentration suggesting that the natural degradative microflora responsible for the observed degradation may acculturate and biodegrade higher contaminant at concentration higher than the concentration that yielded the highest degradation constant.
Key words:*Modelling, diesel, bioremediation, degradation, clay*.

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I. Introduction

Globally, fuel is released into the environment, mostly to soil during transportation and storage due to leaky facilities or accidental spills (Balseiro-Romero *et al*., 2019). Diesel fuel is an energy source also referred as automotive gas oil generated from crude oil distillation process and utilized to effectively power internal combustion diesel engines (Total, 2020). As a petroleum derived product which became major source of energy for homes and industries, its entry into the environments either by accident or negligence comes with adverse effect on humans, plants and animal health (Elechi *et al*., 2018). Moreover, an elevated lung cancer was reported in a cohort study of those exposed to diesel fumes (Howe *et al.*,1983). Although, according to Health Protection Agency (HPA), there is no soil, drinking water and air quality guideline values for diesel. Also, there is no definitive absorption, distribution, metabolism and excretion data due to diesel exposure but the main hazard associated with diesel is chemical pneumonitis that may arise following aspiration of vomitus or inhalation of aerosol or aspiration of liquid during manual siphoning. Moreover, diesel is known to contain substantially neurotoxic substances. Numerous studies have cited acute renal failure (secondary to acute renal tubular necrosis) as a potential complication following acute exposure to diesel (HPA, 2006). It became necessary to identify environment-friendly technology with capability to decontaminate sites polluted by hydrocarbons such diesel fuel.

Bioremediation is considered an environment-friendly soil clean-up technology with significantly lowest adverse effect on soil functional properties, which applies and uses soil organisms such plants, protozoa, bacteria, and/or fungi to degrade soil contaminants (Balseiro-Romero *et al*., 2019). Bioremediation is specifically useful in soil clean-up because the components of hydrocarbons are biodegradable (Ramadass*et al.*, 2018). Modelling of biodegradation of hydrocarbons in soil have been given attention recently.

Several researchers have simulated bioremediation process by developing kinetic and mathematical models capable of predicting these processes (Musa, 2016; Abdulyekeen*et al.*, 2016). Fernández *et al.* (2016) used a mathematical model to describe bioremediation process in clayey soil polluted by diesel using a closed batch reactor. This involves a model which simulate abiotic factors and was also utilised to estimate mass transfer coefficient. Another model simulating mass transfer and biodegradation behaviour was used to estimate biological kinetic and stoichiometric parameters. Both models were validated with experimental data of the previous studies of the authors. Findings showed that the model predictions fitted the experimental data obtained previously. Similarly, Sanni and Emetere (2016) developed a model using a batch process to predict bioremediation process. The outcomes of the simulations using the model compared with the results of an existing model proved the validity and reliability for monitoring the progress of any batch bioremediation process of crude oil polluted soils. Authors concluded that the model can be used to monitor the soil bioremediation by monitoring crude oil residual concentration per time. Ojewumi*et al.* (2017) developed an unsteady state mathematical model based on bulk flow of oil through the soil and molecular diffusion through the pores of the soil. The parabolic partial differential equation developed was resolved into a system of ordinary differential equations by orthogonal collocation method and the necessary boundary condition was used. The simulated data compared with experimental data have validated the developed model. This study is aimed to model the effect of different concentration of contaminant on bioremediation of diesel using the indigenous microorganisms isolated from the soil sample.

II. Materials and Methods

Soil Sampling and preparation

Soil sampling and preparation were explained elsewhere (Clarkson *et al.* 2019). The site for sampling had no history of contamination due to crude oil or any of its products prior to sampling. Sampling was randomly done at the topsoil going few inches below ground surface using a garden trowel. Precisely 10, 000 g of samples were shaken through 4-mm set of sieves and at the end of the particle separation, 6 particle sizes were obtained, and only particles less than 2 mm-size were considered in the manipulation.

Preparation of microcosms

Clay soil were grounded and homogenise for use in the microcosms. 100 ml flasks containing 50 g of the prepared samples were artificially contaminated with 3.0 – 8.0% (w/w) of diesel. Precisely 4% (w/w) of NPK was added to the manipulated samples to stimulate the natural degradation. Deionised water was supplied to facilitate nutrient transfer and all the samples were inoculated with microorganisms isolated from a farmyard. The treatments were prepared in 3 replicates and placed under room temperature in a fume cupboard. The flasks were covered with cotton wool to reduce evaporation and allow adequate aeration in the microcosms.

Microbial inoculation

Microorganisms were isolated from the soil and grown overnight in 100 ml of Tryptic Soy Broth in shakers at 30^oC, and 0.5 ml of the turbid media was washed by a centrifuge at 3000 RPM for 15 minutes and suspended in ¼ Ringer's Solution. The microcosms were inoculated immediately.

Method of hydrocarbon extraction and analysis

At day 7, 14, 21, 28, 35, 42, 49 and 56, flasks were removed, and oven dried at 39^oC for extraction and subsequent analyses. Extraction was done using Soxhlet Extractor and methanol as solvent and the extracts were dried at 38^oC using a rotary evaporator. These were then mixed with known amount of solvent. All samples awaiting analyses were stored in refrigerator at 4 ±1^oC. Residual diesel was analysed by gas chromatography (GC) installed with mass spectrometry.

Estimation of degradation half-life times and residual hydrocarbon using the kinetics

The biodegradation was simulated using the first order kinetics adopted by Agarry*et al.* (2013) in equation (1).

$$C_r = C_o e^{-kt} \quad (1)$$

Where C_r is the residual diesel in soil at time t, C_o is the initial concentration of diesel in soil, k is the biodegradation constant (day^{-1}).

The biological half-life is the time taken for a substance to lose half of its amount which are necessary in environmental modelling and description of the transformation of pollutants (Agarry*et al.,* 2013). The half-life estimation model in equation (2) used *by*Agarry*et al.* is adopted.

$$T_{\frac{1}{2}} = \frac{\ln 2}{k} = \frac{0.693}{k} \quad (2)$$

Microbial Analysis

Viable counts using spread plate method was used to estimate the growth of bacteria in the microcosms. These were carried out at 2, 4, 6 and 8weeks after treatments, by taking out 2 g of samples and suspended it in ¼ Ringer's Solution. Exactly 0.5 ml of the solution was plated on Nutrient Agar and CFUs were isolated using 8-fold serial dilution method. Plates were incubated at 30^oC and counted 48 hours after plating.

Statistical analysis

All data generated were analysed using Two-Way ANOVA in the Microsoft Excel. Similarly, graphs and tables were prepared using the same software.

III. Results and Discussions

Effect of soil manipulation on some chemical and physical properties of soil

The results of some soil properties and soil manipulation have been presented in Table 1. It was shown that, after extracting the particle sizes and manipulated into 6 types, clay soil appears to have the extreme properties. After the soil manipulation, clay have exhibited a pH of 5.22, highest organic matter (14.54mg/g) likely due to high humus particles and lowest porosity/volume of void.

Table 1 Some chemical and physical characteristics of different textures

Soil Texture	**Soil Properties**		**Soil Particles (%)**			
	pH	**Organic matter (mg/g)**	**Clay**	**Silt**	**Sand**	**Porosity**
Clay	5.22	14.54	70	20	10	0.15
Silt	5.89	13.82	87	13	0	0.22
Loam	7.58	12.59	42	41	17	0.52
Silt loam	5.75	13.00	10	70	20	0.39
Loam sand	8.33	9.28	10	10	80	0.41
Sandy loam	8.47	8.07	10	30	60	0.43

Diesel degradation and microbial growth

The results of the degradation of diesel during the 56 days bioremediation experiment are presented in Figures 1 and 2. After 56 days of bioremediation experiment, there were reductions in the concentration of diesel. It can be seen from the two figures that the degradation of diesel in the microcosms have followed similar pattern for all concentrations which did not exceed 5.5% (w/w). Highest decontamination was observed in the microcosm spiked with lowest diesel concentration. Although, lower results were reported previously (Clarkson *et al*., 2019), other unknown factors may have contributed to the degradation. It is difficult to describe the high percent values obtained in the first week for microcosm containing initial diesel concentration of 3% (w/w) because clay particles generally have adverse effect on the degradability of hydrocarbon in soil (Elechi *et al*. 2018).

Generally, cumulative degradation in the microcosms containing initial diesel concentration of 3-5.5% (w/w) ranged between 16-35% in the second week, raised to 19-45% until degradation attained 85% in the 8th week and this was obtained in the sample spiked with 3.5% (w/w). there were low to moderate bacterial growth ($4.2x10^4$ to $3x10^6$ CFU/100ml) throughout the period of remediation. These findings are consistent with the results reported by Haghollahi*et al.* (2016) where much lower degradation was reported after over 200 days bioremediation. It is also consistent with the conclusion of Trindade *et al*. (2005). The lower degradation has been credited to poor aeration and bioavailability. This assertion was upheld in a study conducted by Biswas *et al.* (2017) where acids were used to disrupt the structure of the clay mineral which increases the volume of void and has significantly improved bioremediation. The reduction in contaminant concentration alongside moderate growth of microorganisms suggest that bioremediation was responsible for the depletion of contaminant, probably with little contribution from other abiotic factors.

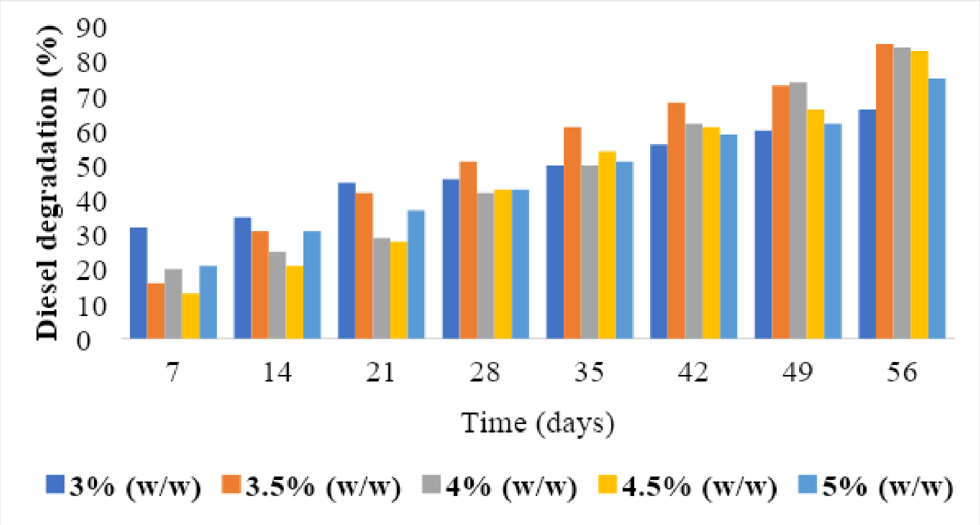

Figure 1: diesel bioremediation in clay soil spiked with concentrations of 3 – 5% (w/w)

Going by the results presented in Figure 2, except at the concentration of 5.5% w/w, the degradation was generally low and right from the first week, bioremediation has been very slow. This could be explained by increased doses of pollutant as this pattern was observed at a pollutant concentration from 6% w/w and at a

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concentration of 8% w/w, biodegradation was slowest. These findings are contrary to the outcomes reported by Sayara*et al.* (2010) where increase in contaminant concentration led to increase in biodegradation of the contaminant. Although, this conforms with the results obtained at successive lower concentrations (3.5-5.5% w/w), it did not conform with the results obtained for higher concentrations which has also been supported by recent findings (Vasilyeva *et al.,* 2020).

It was also observed that there was moderate growth of microorganisms in the first instance of the experiment but at a later period, bacterial growth declined substantially. This may be attributed to the toxicity of the high doses of diesel. Moreover, a two-way ANOVA analysis showed that the effect of concentration of diesel on biodegradation is statistically significant ($P < 0.05$) at 95% level of confidence. The need for toxicity test prior to bioremediation was long documented (Chaineau*et al*., 2003; Nwaichi*et al.,* 2011). In this case, bioaugmentation may be a necessary technique.

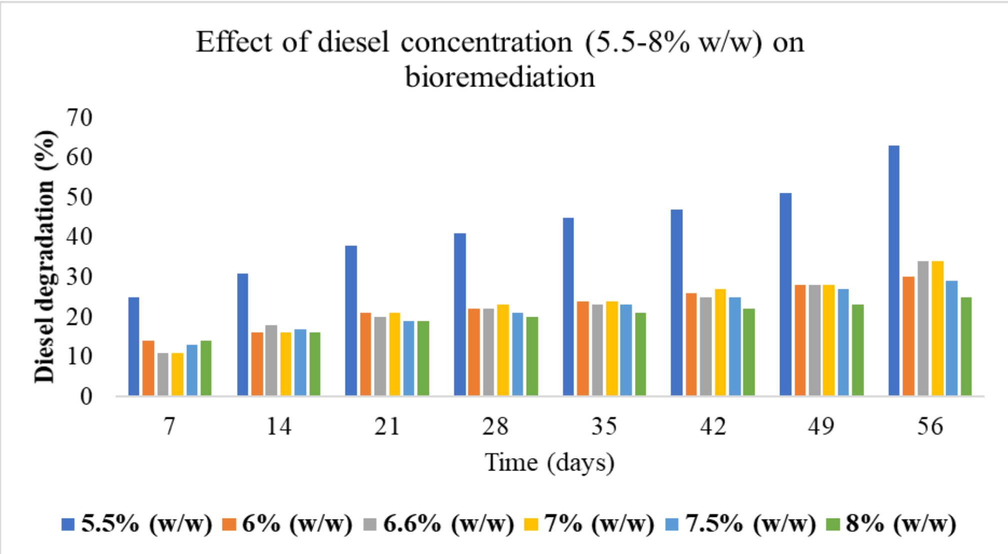

Figure 2: diesel bioremediation in clay soil spiked with concentrations of 5.5 – 8% (w/w)

Biodegradation kinetics and half-life

The first order kinetics model expressed in equation (1) was fitted to a set of experimental data generated to determine the effect of diesel concentration on biodegradation rate. Equally, the half-life periods for oil degradation under these concentrations were also computed using the expression in equation (2). Both results for (k) and ($T_{1/2}$) are shown in Table 2. From the table, the degradation kinetics constants were generally lower and ranged between 0.0082 to 0.0243/day and the half-life ranged from 28.5 to 84.5 days.

Table 2: degradation rate constants and half life times at various concentrations

Treatment Diesel Conc. (mg/Kg)	k (day^{-1})	R^2	$T_{1/2}$ (day)
30000	0.0191	0.9778	36.3
35000	0.0215	0.9722	32.2
40000	0.0227	0.9691	30.5
45000	0.0232	0.9678	29.9
50000	0.0243	0.9649	28.5
55000	0.0191	0.9778	36.3
60000	0.0114	0.9919	60.8
65000	0.0113	0.9919	61.3
70000	0.0107	0.9929	64.8
75000	0.0102	0.9935	67.9
80000	0.0082	0.9958	84.5

It can be seen that highest degradation rate constant (0.0243/day) observed when the concentration was 50000 mg/Kg with a corresponding computed half-life of 28.5 days. It is followed by 0.0232/day at a concentration of 45000 mg/Kg with half-life of 29.9 days. These also suggest that the higher biodegradation rate constant leads to high rate of degradation and resulted in lower half-life at the said concentration. These values are contrary to the findings of Elechi*et al.* (2018) where higher biodegradation rate constants were reported. A possible explanation for this disparity is the differences in the content of clay particles. In Elechi*et al.*, the clay content for the clayey soil was 56.58% and Mangrove soil contained about 31% clay particles while in the present study, the clay particle is 70%. However, similar results were reported by Agarri*et al.* (2015) even though, no information was given by the authors on the particle size distribution of the microcosms.

IV. Conclusion

The main aim of this paper is to model and appraise the effect of contaminant concentration on the bioremediation of diesel contaminated clay soil. This paper confirms that the effect of contaminant concentration on the biodegradation of contaminated soil is significant and there is need for toxicity test while selecting bioremediation strategies to implement.

The kinetics model used was fitted to the experimental data and it has been found that highest degradation rate constant (0.0243/day) and the lowest half-life time were obtained at 5% w/w diesel concentration suggesting that the natural degradative microflora responsible for the observed degradation may acculturate and biodegrade higher contaminant at concentration higher than the concentration that yielded the highest degradation constant.

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