

Forecastin Rate of Biostimulated Bioremediation Using Biodegradation Models

ABSTRACT

There have been several remediation techniques for oil spill-impacted soil in the Nigerian Niger Delta which has not given the much-desired results as the methods used were either inappropriate for the environment or ineffective for the different soil types in the Niger Delta. Bioremediation is a cost-effective and environmentally friendly technology that exploits the capabilities of microorganisms to degrade organic pollutants leading to complete mineralization. It has become the most preferred technique for oil spill remediation on soil in Nigeria. This study is aimed at developing a biodegradation model using biodegradation ratios of a biostimulated biodegradation experiment on crude oil polluted/spiked soil. The model design criteria involve inoculating varying amounts of nutrients (N.P.K fertilizer) into a soil media impacted with crude oil at a ratio of 10kg/kg (10% w/w). The medium for the presentation of the nutrient was water and the volume of water used varied from 30% to 80% saturation. Samples were taken at an interval of about three months to monitor the changes in diagnostic ratios (nC_{17}/Pr , nC_{18}/Ph , $(nC_{17}+nC_{18})/(Pr+Ph)$) using gas chromatography (GC-FID). Results obtained were used to develop a biostimulated biodegradation model to forecast/predict the rate of bioremediation assuming the design considerations are consistent. The model adopted was constrained to the diagnostic ratio $(nC_{17}+nC_{18})/(Pr+Ph)$ which describes the biostimulated biodegradation for all the sample sets. A linear regression model equation, $y=c+bx$ was employed in the model.

Keywords: Biostimulation; diagnostic ratio; soil; Niger Delta; biodegradation model.

1. INTRODUCTION

Biodegradation is a process that has caught worldwide attention, the reason being that biodegraded petroleum hydrocarbon is currently dominating the world petroleum inventory, with the largest biodegraded hydrocarbon reserves neither in the United Kingdom nor the Middle East but on the flanks of the Foreland basin in the Americas [1]. Biodegradation, a process that has hunted petroleum explorations in years past has been recently recognized as a tool that can be harnessed into other aspects of petroleum exploration and production [2]. Biostimulation is an aspect of biodegradation, which involves the periodic supply of nutrients to the microbe colony; this has served as an excellent tool for the remediation of hydrocarbon contamination, specifically on land [3,4].

Bioremediation is of interest worldwide as a potential clean-up option, especially for inaccessible or sensitive environments [5]. A bioremediation technique based on the

optimization of biodegradation has been developed as a soil clean-up technique which is expected to be economical and efficient compared with chemical or physical remediation processes [6,7,8,9].

A model can be defined, as a system of postulates or data and inferences that is presented, as a mathematical or graphical description of an entity or situation that cannot be visualized, but can serve as a set of ideas and numbers that describes the past, present or future of a situation. The basic advantage of generating models that are empirical (experimental) is the potential application of ideas represented by the model or ideas that constitute the model to larger wider cases using the same or similar design considerations. Many studies have been carried out on the modeling of biodegradation for specific conditions involving substrate, specific petroleum hydrocarbon, and specific distribution of microorganism types that consist of the microbe colony of interest. In a classical study Head et al., [1] stated that

significant biodegradation of oil occurs in human time scales, in reservoir models suggest hydrocarbon destruction fluxes in the order of 10^{-4} kg hydrocarbons $m^{-2} yr^{-1}$. Head et al, [1] used an illustration to describe their model which portrayed a decreasing trend of saturated hydrocarbon content. Huang et al. [10] in their study showed a simplified conceptual model for biodegradation for which the concentration of the reactants (substrate) decreases over time represented as distance in depths. Huang et al. [10] also stated the use of ratios such as $(nC17+nC18)/(Pr+Ph)$, $C30\alpha\beta$ hopane/ $(Pr+Ph)$ as reliable parameters for the description of biodegradation trends and assignment of PM (Peters and Moldowan) biodegradation levels. In another, classical study Huang et al. [10] on a generated dynamic biodegradation model indicated compositional gradients of petroleum over the biodegradation time scale, the highly sensitive biodegradation parameter which is $(nC17+nC18)/(Pr+Ph)$ also shows decreasing compositional gradient over time. However, the ratio is highly recommended for use on light to moderately biodegraded samples [10]. Chemlal et al. [11] developed a model using the biopile technique to restore diesel-contaminated soil with a nutrient amendment; after 76 days, the soil was decontaminated with a total petroleum hydrocarbon (TPH) removal rate of about 85%. Agarry et al. [12] used commercial activated carbon (CAC) and plantain peel-derived biochar (PPBC) of different particle sizes and dosages to stimulate petroleum biodegradation in soil using a first-order-kinetic model which shows a positive relationship between the rate of petroleum hydrocarbons reduction and presence of CAC and PPBC in crude oil contaminated soil microcosms. Sarajudeen, [13] used oil and grease content (O&G) and Carbon dioxide (CO_2) released from bioremediation experiments as indicators for monitoring bioremediation in soil contaminated with spent motor oil using a kinetic model. Sample stimulated with NPK (20:10:10) and KH_2PO_4 resulted in a maximum bioremediation response of 75% reduction in initial oil and grease content.

Gogoi et al. [14] developed a model from the result of a laboratory and field pilot test where the effects of aeration, nutrients, and inoculation of extraneous microbial consortia on the bioremediation process were investigated. The field pilot test showed that up to 75% of the hydrocarbon contaminants were degraded within 1 year; the moisture content in each cell was maintained at 50 and 65% during the entire

period. Monitoring the success of the bioremediation experiment was done by estimating from time to time the oil content, pH, moisture content, Nitrogen, and Phosphorus levels along with the total microbial content of the soil samples. Dave et al. [15] developed a sequential process for the remediation of oil-contaminated soil which includes washing, absorption of oil from water using peat, and bioremediation of contaminated peat. This was followed by the development of a biodegradation model which was used to calculate the time required for the complete degradation of the contaminated soil. Total degradation of the oil was achieved in 68.5 days.

This work involves developing a model using biodegradation ratios of a biostimulated biodegradation experiment on crude oil-polluted soil with model design criteria, which can be used to monitor and forecast the rate of soil biodegradation.

2. MATERIALS AND METHODS

A bulked clean soil sample was impacted with crude oil at a ratio of 10g/kg (10%w/w) as described by Agarry and Ogunleye [16] and Ubochi et al. [17]. One hundred and fifty grams (150g) of the contaminated soil was placed in each of the seven microcosms (bioreactors) in an aerobic condition at an average temperature of 30 °C.

The laboratory samples were subjected to different conditions that foster biodegradation of the hydrocarbon compounds inherent in the matrix of the soil (substrate). The substrate in this context is the medium in which the microorganism lives. The conditions were constrained to the variability of the concentration of nutrients inoculated into the media (bioreactors) and the medium of presentation of the nutrient inoculants. The nutrient was specifically NPK (Nitrogen-Phosphate-Potassium) fertilizer, the medium for the presentation of the nutrient was water and the volume of water used varied from 30% to 80% saturation while the mass of the nutrient was constant (30g) over a variable volume of water. No cultured series and specific microorganisms were introduced into the media (substrate) for all samples, the biodegradation media, which was soil, implies that only the microorganisms present in the substrate were allowed to act on the substance (hydrocarbon undergoing degradation). The process of the

addition of nutrients to enhance biodegradation is called biostimulation. The sample codes are as follows: A – Crude oil + 30g NPK + Soil; B – Crude oil + 60g NPK + Soil; C – Crude oil + Soil (Control); D – Crude oil + 80g NPK + Soil; E – Crude oil + 30g NPK + Soil + 30% H₂O saturation ; F – Crude oil + 30g NPK + Soil + 50% H₂O saturation; G – Crude oil + 30g NPK + Soil + 80% H₂O saturation.

Samples were taken at an interval of about three months to monitor the changes in diagnostic ratios (nC_{17}/Pr , nC_{18}/Ph , $(nC_{17}+nC_{18})/(Pr+Ph)$). Results obtained were used to develop a biostimulated biodegradation model to forecast/predict the rate of bioremediation assuming the conditions used in the experiment. The Linear regression model equation is thus: $y=c+bx$;

Where; y is the parameter/biodegradation ratio, b is the rate of biodegradation, x is the biodegradation times and C is a constant.

2.1 GC Analysis

Ten grams (10g) of the soil sample was blended with 10g of anhydrous sodium sulfate and extracted in a soxhlet apparatus for 4 hours. The extract was later concentrated to 2ml with a rotary evaporator. The concentrated extract was fractionated using activated silica gel of 100 mesh size topped with 0.5g Aluminum Oxide (activated). The column was eluted with 20ml n-hexane to obtain the aliphatic fraction which was later concentrated to 1 ml in a rotary evaporator. The aliphatic hydrocarbons were determined using a Gas Chromatograph (Agilent 6890N) with an HP-5 fused silica column of dimensions 30m×250µm×250 µm film thickness and 5% phenyl methyl siloxane capillary column. The oven temperature program was maintained at 40°C for 2min and then increased at a rate of 10°C/min until a final temperature of 320°C was reached. The final temperature was held for 2

min with Nitrogen carrier gas held at a constant flow rate of 2.6ml/min and pressure of 10.4psi [18].

3. RESULTS AND DISCUSSION

In this study, biodegradation models are generated based on sample sets A, B, C, D, E, and F. Each sample set was exposed to a specific substrate treatment regarding the concentration of nutrients and water content over the same time frame. Biodegradation parameters used were $(nC_{17}+nC_{18})/(Pr+Ph)$, nC_{17}/Pr , and nC_{18}/Ph .

The best-fit line was generated for each plot and the equation was used to derive the slope which describes the rate for the process at all-time throughout the process.

The models were generated for all sample sets and all stated parameters using the data in Table 1. The models show that the biodegradation/parameter ratio, $(nC_{17}+nC_{18})/(Pr+Ph)$ was consistent for all sample sets as shown in Figures 1 to 4. On the bases of possible variants of the trends for nC_{17}/Pr , and nC_{18}/Ph , the most plausible model was therefore constrained to the $(nC_{17}+nC_{18})/(Pr+Ph)$ parameter, which describes the biostimulated biodegradation for all sample sets.

Figs. 5-8 depict the biodegradation models for the $(nC_{17}+nC_{18})/(Pr+Ph)$ parameter of the soil sample sets.

The correlation coefficient (R^2) of the models for the sample sets varies from 0.7785 to 0.9995; sample sets F and G have the lowest correlation coefficient of 0.8767 and 0.7785 respectively. The correlation coefficient indicates the relationship between the variation in the parameter ratio compared to the time that elapses during the experiment.

Table 1. Biodegradation ratios obtained from the laboratory study, 1,2,3 represents the months-June; of August; November

Please check this table

SET A	nC_{17}/Pr	nC_{18}/Ph	$(nC_{17}+nC_{18})/(Pr+Ph)$
1	0.7	0.7	0.7
2	0.2	0.7	0.3
3	0.0	0.0	0.0
SET B	nC_{17}/Pr	nC_{18}/Ph	$(nC_{17}+nC_{18})/(Pr+Ph)$
1	0.6	1.3	0.7
2	0.3	0.8	0.4

3	0.1	0.3	0.1
SET C	nC17/Pr	nC18/Ph	(nC17+nC18)/(Pr+Ph)
1	0.5	0.3	0.5
2	0.4	0.3	0.4
3	0.4	0.1	0.3
SET D	nC17/Pr	nC18/Ph	(nC17+nC18)/(Pr+Ph)
1	1.4	1.1	1.4
2	0.7	0.2	0.5
3	0.3	0.2	0.2
SET E	nC17/Pr	nC18/Ph	(nC17+nC18)/(Pr+Ph)
1	0.4	0.6	0.5
2	0.4	0.1	0.3
3	0.3	0.0	0.2
SET F	nC17/Pr	nC18/Ph	(nC17+nC18)/(Pr+Ph)
1	2.6	0.9	2.5
2	0.6	0.2	0.5
3	0.1	0.2	0.1
SET G	nC17/Pr	nC18/Ph	(nC17+nC18)/(Pr+Ph)
1	2.2	1.0	2.1
2	0.7	0.3	0.6
3	0.6	0.0	0.6

Source: [18]

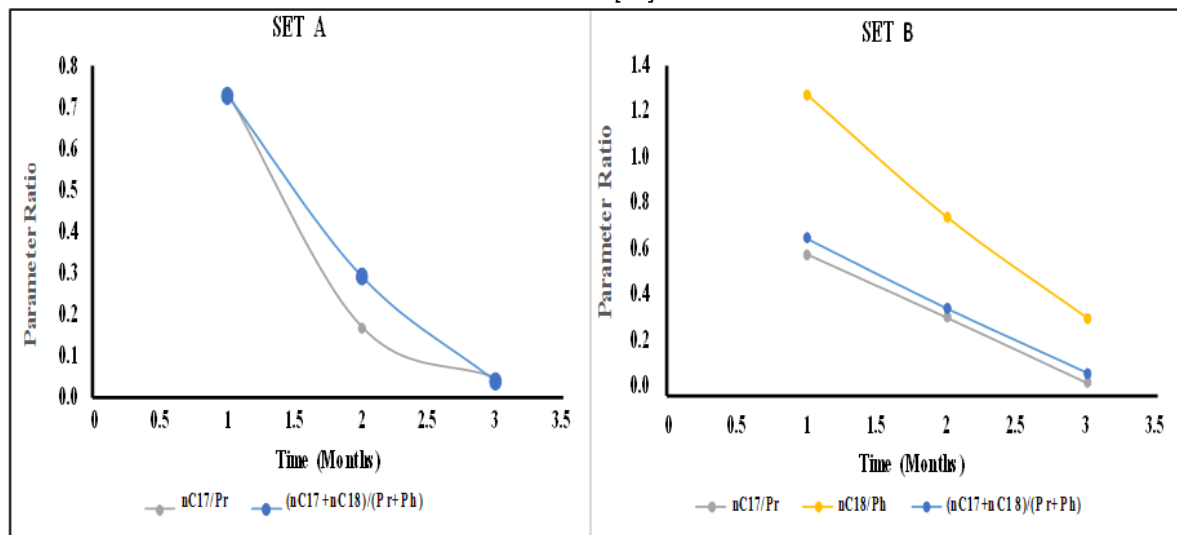


Fig. 1. Biostimulated biodegradation profile for sample sets A and B

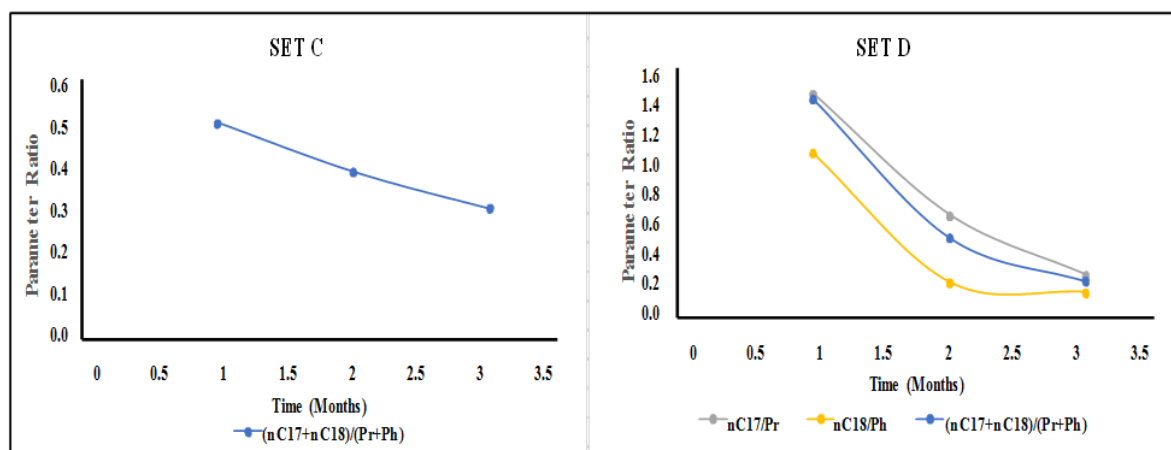


Fig. 2. Biostimulated biodegradation profile for sample sets C and D

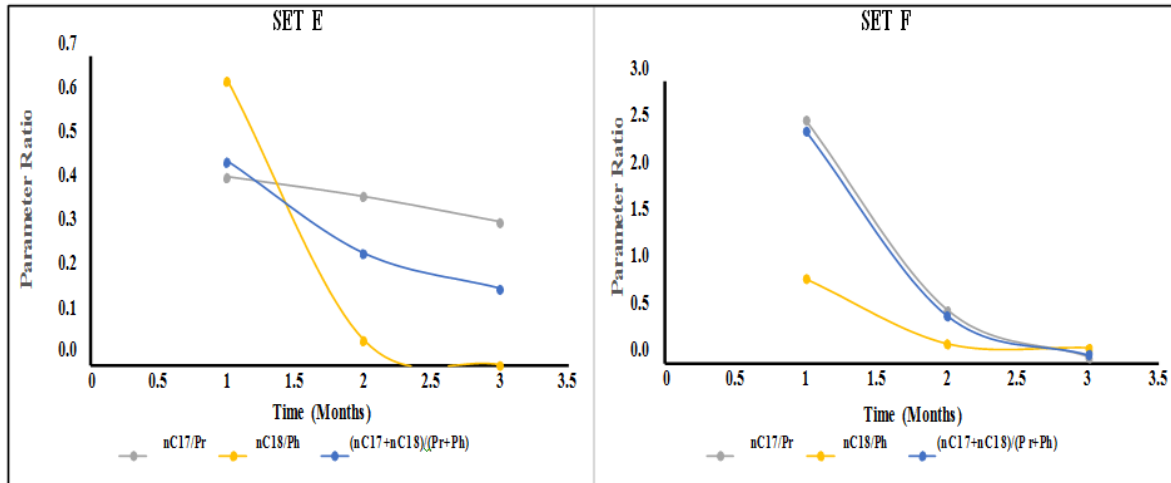


Fig. 3. Biostimulated biodegradation profile for sample sets E and F

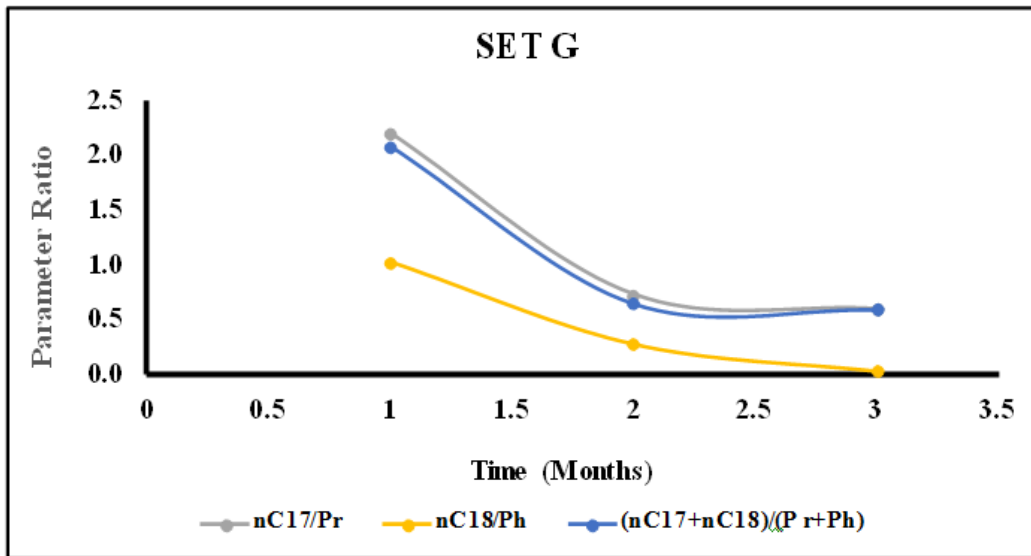
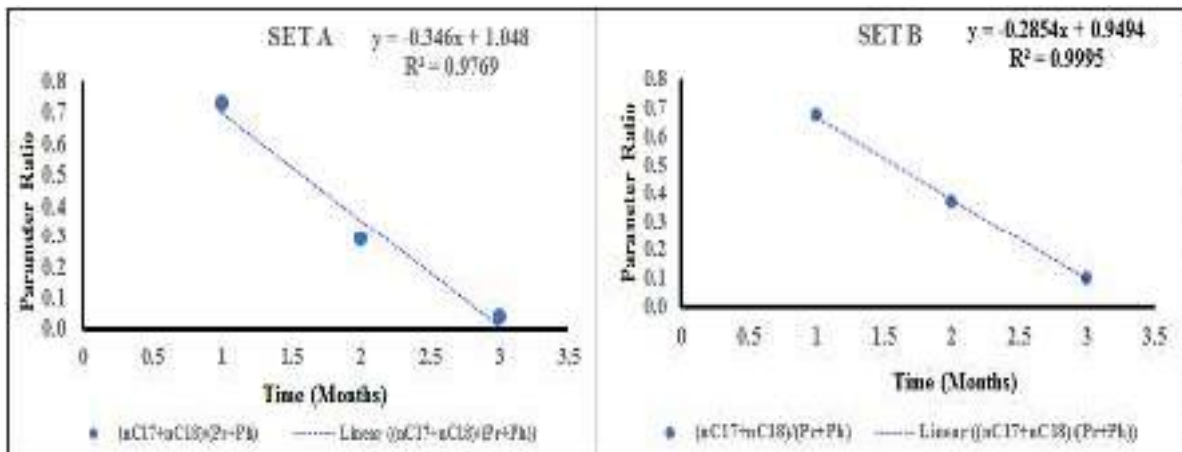


Fig. 4. Biostimulated biodegradation profile for sample sets G



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Fig. 5. Biostimulated biodegradation models for samples set A and B

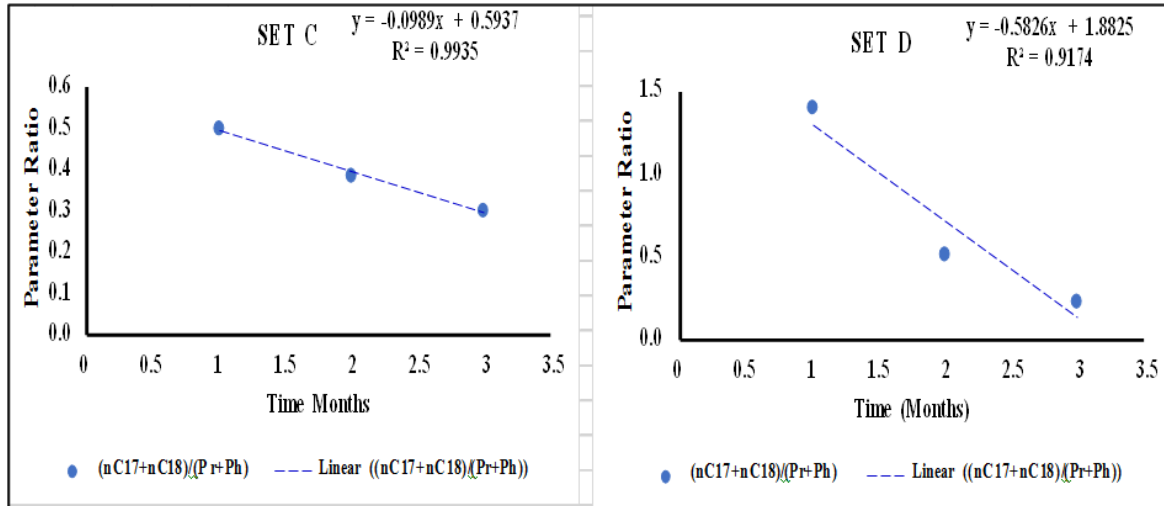


Fig. 6. Biostimulated biodegradation models for samples set C and D

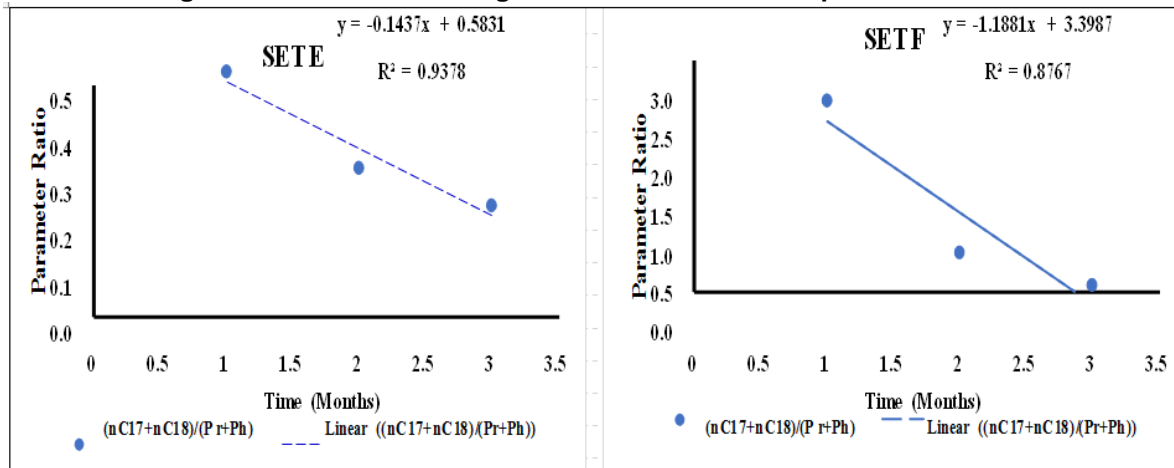


Fig. 7. Biostimulated biodegradation models for samples set E and F

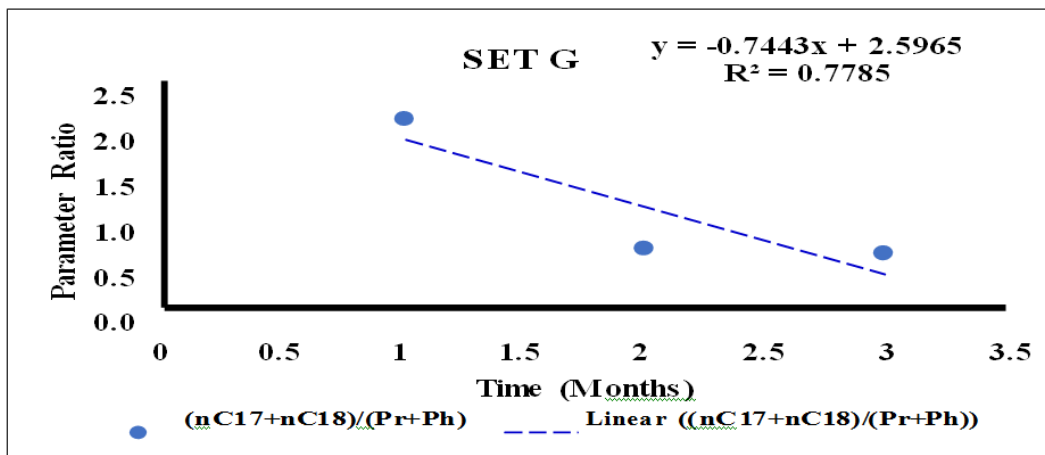


Fig. 8. Biostimulated biodegradation model for samples set G

All the correlation values for the sample sets indicate a good positive relationship to an excellent positive relationship. It can be inferred that sample sets F and G had a good positive correlation coefficient between the parameter ratio $[(nC17+nC18)/(Pr+Ph)]$ and time, while other sample sets had an excellent positive correlation coefficient between the parameter ratio and time. The rates as indicated by the slope could be expressed as $[(nC17+nC18)/(Pr+Ph)] / [Time]$. The rate varies from 0.09/3 months to 0.74/3months. The lowest of the rates (0.09/3 months) is that of sample set C (which is the control) without any application of nutrients as shown in Figure 6. The control was subjected to the natural biodegradation process. However, sample set F had the highest rate (1.18/3months) as shown in Fig. 7, this may infer that for water-sediment systems that model is the best given the specific microbes colony, specific oil, and soil. The negative sign of the slopes in the equation refers to the decrease in the parameter ratio too much lower values throughout the experiments. The trend of the profiles did not show any significant difference that would have significantly discriminated the control from others, even the total aliphatic hydrocarbon left in the soil after the expiration of the period for the biostimulation experiment. The dip of the best-fit line is high for high rates and low for low rates.

It was also observed that sample sets with high biostimulated biodegradation rates as derived from the equation of the best-fit line for the generated models indicated that the targeted zero parameter ratio value was attained before the 9th month (3 months). Other sample sets did not attain the zero values or attained the zero values after the 9th month, which is the time frame for the experiment.

3.1 Forecasting Biostimulated Bioremediation

Forecasting the time at which the substrate will be completely degraded can be achieved using the models generated. The repeatability of the experiment keeping to specific routines is critical; this provides the basis for which the models can be applied to other situations/field sites. The specific condition, which entails nutrient inoculation, and water saturation, represents the design considerations, while each sample set represents a model. Any model could be employed for any site depending on the site characteristics whether nearshore coastline, wetland, floodplains, and intertidal sediments with different water saturation levels and

sediment content or onshore on dry land, moist land with different moisture content, which reflects and corresponds to the desired design consideration of the model. Once the initial parameter ratio is known, assuming the rate is constant for a particular model and its design consideration, the period can be projected and forecasted.

A practical example of forecasting is the model for sample set C (Fig. 6), which shows that the parametric ratio was not reduced to zero at the end of the experiment, however, based on the model, the time at which the parameter ratio will attain zero can be forecasted. Sample set C had the parameter ratio at about 0.3 at the end of the experiment, however on the forecast, it is observed that it will require 9 (nine) more months to reduce the parameter ratio to zero. This infers that it will take nine more months to completely or near completely degrade the aliphatic hydrocarbon using the same model, which implies the same site characteristics or design consideration of the model.

Similarly, sample set E had a parameter ratio of 0.2 at the end of the experiment, however, it was forecasted based on the same model and design considerations or the same site characteristics to require 3 (three) more months for the parameter ratio to reduce from 0.2 to zero or near zero, implying, that it will require three more months for complete or near complete degradation of the aliphatic hydrocarbons.

Modeling biostimulated remediation is an aspect of biodegradation, which is an evolving science, and several models are being generated depending on the diversity of the environment and the occurrences of spills of varieties of hydrocarbon nature, the evolution of more active nutrients determines the level of advancement over time.

Modeling biostimulated remediation has its application in diverse areas such as heavy oil exploration and production, remediation of spill sites, decommissioning of well sites, reclamation of land areas, microbial-enhanced oil recovery, etc.

4. CONCLUSION

The models are the graphs and equations describing the biodegradation dynamics of the system, which can also be used for forecasting rate and time as a function of the design considerations.

The best parametric ratio is $(nC_{17}+nC_{18})/(Pr+Ph)$, its profile was consistent for all sample sets spanning across different design considerations. The slowest rate (0.09/3months) is that of sample set C (which is the control), while the fastest rate is (1.18/3months) for sample set F.

A forecast for sample set E is that it will take up to 4 months for the biodegradation to achieve a zero of $(nC_{17}+nC_{18})/(Pr+Ph)$, implying that it will take up to 4 months to completely exhaust/remove nC_{17} and nC_{18} alkanes relative to Pr and Ph.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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