Use Of Biodegradation Ratios In Monitoring Trend Of Biostimulated Biodegradation In Crude Oil Polluted Soils

Okorondu, J.N., Osuji, Leo C., Ofodile, S.E.

Abstract: This study deals with biodegradation experiment on soil contaminated with crude oil. The soil sample sets A, B,C D, E, F, G were amended with inorganic fertilizer to enhance microbial growth and hydrocarbon degradation, moisture content of some of the sets were as well varied. Biodegradation ratios (nC_{17}/Pr, nC_{18}/Ph and (nC_{17}+nC_{18})/(Pr+Ph) were used to monitor biodegradation of soil sets A, B,C D, E, F, G for a period of 180. The soil samples were each contaminated with the same amount of crude oil and exposed to specific substrate treatment regarding the amount of nutrients and water content over the same period of time. The trend in biodegradation of the different soil sample sets shows that biodegradation ratio (nC_{17}+nC_{18})/(Pr+Ph) was more reflective of and explains the biodegradation trend in all the sample sets throughout the period of the experiment, hence, a better parameter ratio for monitoring trend of biostimulated biodegradation. The order of preference of the biodegradation ratios is expressed as nC_{18}/Ph < nC_{17}/Pr < (nC_{17}+nC_{18})/ (Pr+Ph). This can be a relevant support tool when designing bioremediation plan on field.

Keywords: biodegradation ratios, biostimulated biodegradation, trend, crude oil, soil

1.0 Introduction

Crude oil and its derivatives, as the key energy generating substances and raw materials used for production, are very widely used in all domains of work and everyday life [10][5]. Both soil and water become contaminated by oil derivatives due to accidental spill in their exploitation, transportation, processing, storing and utilization as well as sabotage. [10][5]. Oil contamination in Ogoniland is widespread and severely impacting many components of the environment. Most of the clean-up technologies which have been employed in the remediation of oil contaminated soil in Ogoniland have not yielded excellent results [9]. The aim of this research is to monitor the biodegradation trend in laboratory experiment using biodegradation ratios based on sample sets, A, B,C D, E, F, G, which could be applied on a field scale for different scenarios, which reflects dry land (soil) environment and sediment−water systems, such as coastal, nearshore, wetlands, lowland and other flood prone land environments.

2.0 Materials and methods

This laboratory scale experiment was carried out under controlled conditions. The variables include; nutrients (inoculants) and moisture content. Round shaped stainless steel trays of 87.67cm circumference was used as bioreactors. The bulked clean soil samples was impacted with crude oil at a ratio of 10g/kg (10%w/w) as described by [7][8]. 150g of the contaminated soil was placed in each of the seven microcosms in an aerobic condition at an average temperature of 30^0C.

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The crude-oil contaminated soil in each of the 3 trays was amended with different amounts (30g, 60g, 80g) of N.P.K fertilizer (15.15.15) as similarly described by [5][8]. Also, the 4^{th}, 5^{th} and 6^{th} trays were maintained at 30%, 50%, and 80% water saturation. The fourth tray (Sample C) served as a control with no nutrient amendment. All bioreactors were manually mixed each day to enhance oxygenation and kept moist during the 180 days experimental period. The biostimulation process was carried out on the basis of sample sets, each soil set was subjected to the same period of time and samples were obtained at the same intervals and analysis for aliphatic hydrocarbons were carried out to know the extent of biodegradation. The soil sample sets were three, the June, August and November sample sets, these are the times when soil samples were harvested for analysis; which forms the bases of comparisons. 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% H_2O saturation
F – Crude oil + 30g NPK + Soil + 50% H_2O saturation

Ten grams (10g) of the soil sample was blended with 10g of anhydrous sodium sulphate and extracted in a soxhlet apparatus for 4 hours. This 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 anhydrous sodium sulphate. 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 HP-5 fused silica column of dimensions 30m×250µm×250 µm film thickness and 5% phenyl methyl siloxane capillary column. The oven temperature programme was maintained at 40^0C for 2min and then increased at a rate of 10^0C/min until a final temperature of 320^0C 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.

Determination of moisture content

The moisture content of the soil samples was determined using gravimetric method. The oven drying methods followed standard methods prescribed by [1] A. Each sample (5 grams) was weighted using a Denver Balance (Model No. TP 214) to the nearest 0.0001g, and the weight was recorded.

3.0 Results and discussion

3.1 Biodegradation of petroleum hydrocarbons

The process adopted in this study was biostimulation, which is the application of nutrient to micro-organism for sustenance and enhance degradation of the hydrocarbon components, [3][7][6][2]. The following observations were made on the laboratory samples: Considering sample set A, fig. 1, which shows that the biodegradation ratio decreases from June to November. This implies that composition of the media and the inoculation of nutrients to the substrate favors the biodegradation; however, it is observed that the nC_{18}/Ph ratio did not show regularity in the trend of the ratio as represented by nC_{17}/Pr and nC_{17}+nC_{18}/ (Pr+Ph). The isopreniod compound Pristane is present up to 300mg/kg in all three samples. The samples for set B in fig.2 shows similar trend as the samples for set A, however set B samples show higher concentrations of hydrocarbon for the respective intervals of harvest of samples from the media for biodegradation monitoring. The fact that sample B have lower concentration of the isoprenoid compound Pristane in November as shown in fig.2 might indicate that the media for sample set A may be less effective compare to that of sample set B. or there could have been incorporation from biogenic sources, the isopreniod compound Pristane is present up to 250ppm in all three samples. The sample for set C(fig.3) shows some irregularities, the trend of biodegradation is not consistent. However, the trend for concentration of the hydrocarbon components in the sample sets show that concentrations of the components decreases with the time lapse, inferring decrease in concentration with increase in time of biodegradation, though the parameters do not show the expected trend. This may be attributed to inconsistency in the biodegradation of components used for the parameter, i.e. if the biodegradation of the aliphatic hydrocarbons is inconsistent with the isopreniods, the ratio may show inconsistency in the trend reflecting the time interval at sample harvest for monitoring. However, samples sets A and B shows a more consistent trend with significant present of hydrocarbon in the November sample compared to the C sample set for November. The isopreniod compound Pristane is significantly (1200mg/kg) present in the June and August samples.

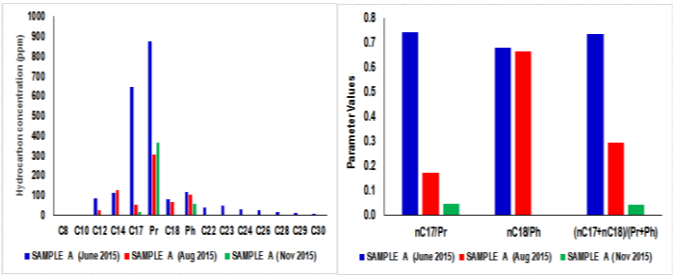

Fig.1.*The clustered column plot representing set A laboratory studies*

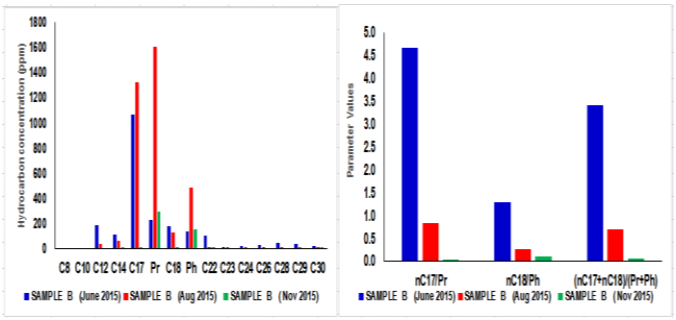

Fig.2.*The clustered column plot representing setB laboratory studies*

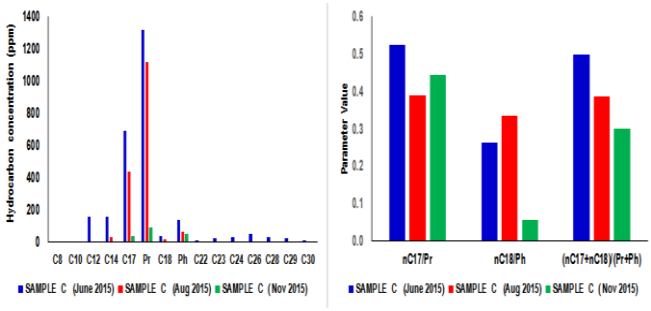

Fig.3.*The clustered column plot representing setC laboratory studies*

The set D samples (fig.4) show a trend that portrays significant biodegradation, the only abundant hydrocarbon components are nC_{17} and Pristane. The clustered column plots also show near similar trend for all three biodegradation ratios. This indicates that the composition of the media was effective in enhancing biodegradation. The isopreniod compound Pristane is only significantly present (1600mg/kg) in the June sample, but less than 160mg/kg August sample and about 100mg/kg in November sample. The trend for sample set E (fig. 5), show significant presence of hydrocarbon components in the November sample. The media does not seem to enhance biodegradation; though the concentrations of hydrocarbon components is much lower relative to the trend of sets A and B but is similar to that sample set A and B. The isopreniods Pristane and Phytane are significantly present. Phytane is near absent in sets A (56mg/kg), B (50mg/kg), C (52mg/kg) and D (60mg/kg) for the November samples, but significantly present in set E (300mg/kg) for the November sample. The isopreniods are more recalcitrant and resistant to biodegradation; their significant present may imply less effective media for biodegradation. The set F (fig.6) samples have similar trend with set E samples, the isopreniods compounds, Pristane and Phytane are present in significant concentrations. The compound Phytane in the sets are thus: Set A (56mg/kg), B (50mg/kg), C (52mg/kg), D (60mg/kg) and E (269.1mg/kg) for the November, that of set F is 441mg/kg in the November sample. The media for the set F samples is less effective for biodegradation, relative to other media based on isopreniods. The set G samples (fig.7) show a trend that is fairly similar as to that of sets A and B as shown in figure 4.14. The isopreniod Pristane is present but relatively higher and significant concentration of 1600mg/kg in the November

sample. However, there is significant absence of higher hydrocarbon components.

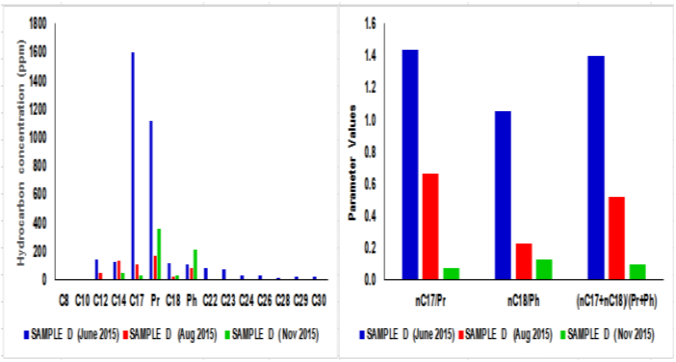

Fig. 4.*The clustered column plot representing setD laboratory studies*

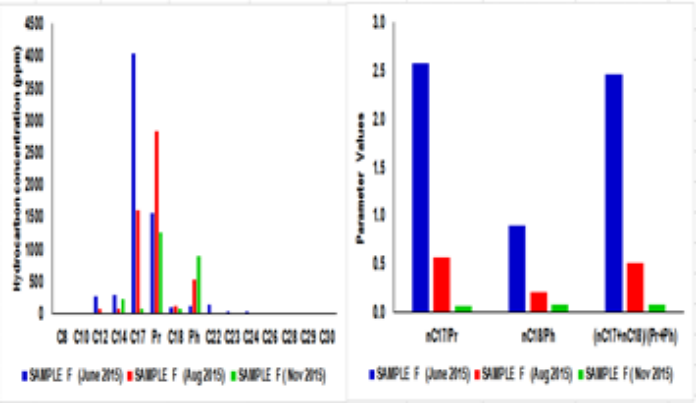

Fig. 5.*The clustered column plot representing setE laboratory studies*

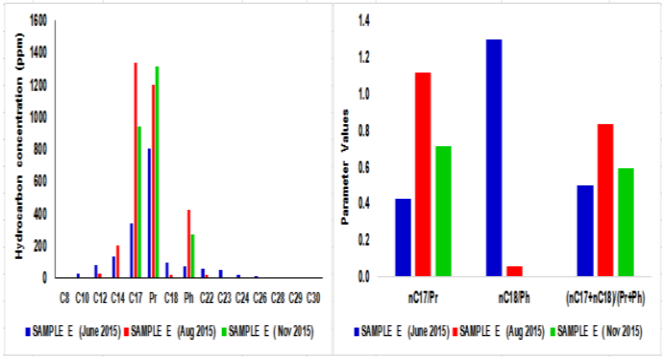

Fig. 6*.The clustered column plot representing set F laboratory studies*

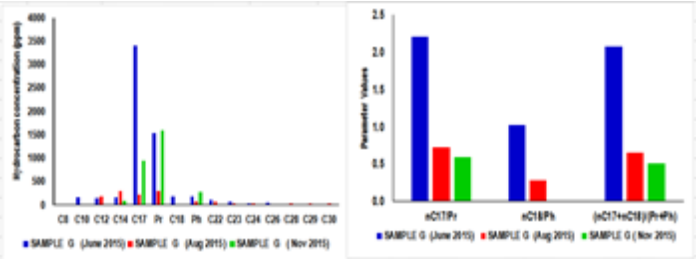

Fig. 7.*The clustered column plot representing set G laboratory studies*

Table. 1.0. *Biodegradation ratios obtained from the laboratory study, 1,2,3 represents the months-June, August, November*

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	nC_{17}/Pr	nC_{18}/Ph	$(nC_{17}+nC_{18})/(Pr+Ph)$
1	0.5	0.3	0.5
2	0.4	0.3	0.4
3	0.4	0.1	0.3
SET D	nC_{17}/Pr	nC_{18}/Ph	$(nC_{17}+nC_{18})/(Pr+Ph)$
1	1.4	1.1	1.4
2	0.7	0.2	0.5
3	0.3	0.2	0.2
SET E	nC_{17}/Pr	nC_{18}/Ph	$(nC_{17}+nC_{18})/(Pr+Ph)$
1	0.4	0.6	0.5
2	0.4	0.1	0.3
3	0.3	0.0	0.2
SET F	nC_{17}/Pr	nC_{18}/Ph	$(nC_{17}+nC_{18})/(Pr+Ph)$
1	2.6	0.9	2.5
2	0.6	0.2	0.5
3	0.1	0.2	0.1
SET G	nC_{17}/Pr	nC_{18}/Ph	$(nC_{17}+nC_{18})/(Pr+Ph)$
1	2.2	1.0	2.1
2	0.7	0.3	0.6
3	0.6	0.0	0.6

4.0 Conclusion

Biostimulated biodegradation laboratory studies were carried out on crude oil contaminated soil samples. The biodegradation ratios considered were nC_{18}/Ph, nC_{17}/Pr, and $(nC_{17}+nC_{18})/(Pr+Ph)$. The $(nC_{17}+nC_{18})/(Pr+Ph)$ ratio best explains the biodegradation profile for most of the sample sets. This invariably infers that $(nC_{17}+nC_{18})/(Pr+Ph)$ ratio is the most preferred of the suite of ratio used for monitoring degradation trend for biostimulated biodegradation. The order of preference of the biodegradation ratios is expressed as $nC_{18}/Ph < nC_{17}/Pr < (nC_{17}+nC_{18})/(Pr+Ph)$. The study infers that biostimulation during biodegradation studies enhances the rate of the degradation process, though there may be contributions from integrated interplay of other factors.

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