



# **Surfactant Enhanced Bioremediation of Hydrocarbon Contaminated Soils Using Alkyl Polyglucoside**

**C. E. Ezekiel<sup>1</sup>, Leo C. Osuji<sup>1,2</sup> and M. C. Onojake<sup>1,2\*</sup>**

<sup>1</sup>*Africa Centre of Excellence in Oilfield Chemicals Research, University of Port Harcourt, P.M.B. 5323, Choba, Port Harcourt, Nigeria.*

<sup>2</sup>*Department of Pure and Industrial Chemistry, University of Port Harcourt, P.M.B. 5323, Choba, Port Harcourt, Nigeria.*

## **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## **ABSTRACT**

Bioremediation is an efficient and environmentally friendly method for the degradation of petroleum hydrocarbons in contaminated soils. This study investigated the effects of biosurfactant alkyl polyglycosides (APG) on enhanced biodegradation of petroleum hydrocarbon contaminated soils. Three soil samples were contaminated with two different grades of crude oil (medium and Light). Alkyl polyglucoside was synthesised and subjected to FTIR for confirmation of the product before it was applied in the remediation of contaminated soil. The alkyl polyglucoside is used as a treatment regime in the remediation of the hydrocarbon contamination in the three soil samples. Results of total petroleum hydrocarbons (TPH) before remediation with bio-surfactant showed that samples contaminated with medium crude for Eneka, Ozuoba and Rukpokwu were 15744.00 mg/kg, 11359.00 mg/kg and 11470.00 mg/kg respectively and after remediation reduced to 4276.00 mg/kg, 4265.00 mg/kg, and 3205.00 mg/kg, showing a reduction percentage of 72.84%, 62.44% and 72.05% respectively. Soil samples contaminated with light crude showed result of TPH of 11339.00 mg/kg, 10662 mg/kg and 10226 mg/kg and after remediation reduced to 2981 mg/kg,

\*Corresponding author: E-mail: ononed@yahoo.com, mudiaga.onojake@uniport.edu.ng;

3879 mg/kg, and 4245 mg/kg respectively showing a reduction percentage of 73.71%, 63.62 % and 58.49% respectively. The enhanced efficiency of the bio-surfactant at degrading total petroleum hydrocarbons was achieved as a result of the increased solubility thus improving the bioavailability of the hydrocarbons due to the action of the alkyl polyglucoside.

*Keywords: Bioremediation; petroleum hydrocarbon; contaminated soil; bio-surfactant; alkyl polyglucoside; bioavailability.*

## 1. INTRODUCTION

Industrial activities impact the environment in several ways, sometimes this impact can be negative. The petroleum industry is not an exception, amidst all the benefits from the industry, the release of petroleum hydrocarbon to the environment leading to the introduction of hazardous contaminants to the environment is one of such negative impact of the petroleum industry [1]. The release of these chemicals occurs as a result of equipment failure, accidents, poor operational practices/ conducts, inappropriate use and disposal and at other times sabotage, during the drilling, processing / refining, transportation and storage stages [2,3,4].

Petroleum hydrocarbon are toxic and contain contaminants that cause varying degrees of harm to the environment, human and animals [5,6,7]. Hydrocarbon spill on land affects the soil adversely, in some instances causing the alteration of the soils physical and chemical properties [8,6]. A number of factors determine the extent of damage done to the soil, these factors include – soil type, the hydrocarbon composition, the quantity of hydrocarbon spilled and the length of time of exposure of the soil to the contaminants [9]. Due to the presence of these chemicals, the soil's ability to support plant growth is inhibited, leading to stunted growth, there is also poor/deformed root development, deformed flowers etc. The overall result is poor yield [10]. The presence of petroleum hydrocarbon in soil also introduces heavy metals which can bioaccumulate and bio- magnify leading to severe health challenges.

Soil contamination by petroleum hydrocarbons is the most severe environmental issue in oil producing regions of the world. As these products are spilled on land, it migrates vertically through the soil pores to the sub surface sometimes reaching the ground water [11]. During this migration, these hydrocarbons exist differently from the aqueous phase. This is as a result of the low solubility of these contaminants.

The phase in which they exist is called the Non-aqueous phase liquid (NAPL). NAPL are phase liquids and do not readily dissolve in water due to their hydrophobic nature [12].

In a bid to eliminate or reduce the harmful effects of these contaminants, remediation action is usually undertaken in order to restore the soil to its original state or render the contaminants harmless [13]. Several remediation approaches and methods have been studied and successfully applied in the remediation of contaminated sites with varying degrees of effectiveness; other has however shown different limitations [6,14,15].

Bioremediation of petroleum hydrocarbon contaminated sites has been shown severally to be a viable method in the removal of these contaminations. This method uses microorganisms such as bacteria and fungi to degrade the petroleum hydrocarbons contaminants to simpler substrates that are non-toxic. Bioremediation is the most frequently and commonly used mainly for its ability to eliminate the target chemicals without producing toxic by-products, its less technological involvement among other advantages [16,17].

According to Ossai et al. [6], the method has the advantage of being environmentally friendly, facile and cost-efficient. Notwithstanding its viability, bioremediation is influenced by a number of factors amongst which are nature of hydrocarbon, nature soil, virility of the soil, duration of exposure of the soil to the contaminants [18]. Thus, when adopting this method, it is important to study these limiting factors that can affect the efficacy of the process. Lower molecular weight hydrocarbons (aliphatic hydrocarbons) are degraded easily by microorganisms whereas the higher molecular weight long chain hydrocarbons, long chain, branched or cyclic chain hydrocarbons are no readily degraded by these microorganisms [18]. Bioavailability is a critical factor for consideration when opting for any bioremediation technique [19,20,21,22]. Bioavailability is the amount of contaminant that can be accessed by the

microorganisms that biodegrade these compounds. It is the tendency of the hydrocarbon to be available for take up by the microorganism. Owing to the hydrophobic nature of petroleum hydrocarbons and their low solubility, bioavailability is also low. Bioavailability, according to Vijakumar and Saravanan [23], involves increasing the solubility of hydrocarbons making it more accessible for microorganisms, as their low water solubility limits their availability to microorganisms. Surfactants are the main agents responsible for this, as a result of the presence of both hydrophobic and hydrophilic groups which effectively alters the oil-water interface. Surfactants have been shown to act on the soil-water interface and the water-microbial interface improving the desorption of petroleum hydrocarbon from the soil enhancing the degradation by microorganisms [24].

Bioremediation techniques used for the remediation of hydrocarbon contaminated soil, must consider the hydrophobic nature of the contaminants (which reduces greatly their availability to microorganisms), effects of hydrocarbon weathering in soil/sediment, and the poor biodegradability of high-molecular weight hydrocarbons. Petroleum hydrocarbons are hydrophobic solids and thus are difficult to breakdown due to their low water solubility and bioavailability [25]. The use of surfactants has however been shown to enhance bioavailability [26,27]

Two different modes are possible. Surfactants can disperse the oil in the aqueous phase (solubilization) and thus make it available for biodegradation, or they can make the oil flow by lowering the surface tension (mobilization; [27]).

The initial phase involves the rapid removal hydrocarbon due to the high availability, and a second phase which occurs after the bioavailable hydrocarbon has been removed, this phase is longer and uses mass transfer mechanisms (desorption and diffusion). Many conventional surfactants however, are highly toxic [27], hence, the use of eco-friendly polymeric surfactant from bio degradable materials [24] in this study.

In this work, Petroleum hydrocarbon contamination was remediated using the increase in bioavailability approach which was achieved by the addition of a bio-friendly non-ionic surfactant (prepared from renewable raw

materials – glucose and fatty alcohol in the presence of an organic catalyst) on three different soil samples spiked with two crude oil samples (light and medium crude oil samples). The surfactant used in this work is an Alkyl polyglucoside (Decyl polyglucoside). Alkyl glucosides are non-ionic biodegradable polymeric surfactants with glucoside head group which is prepared using glucose and fatty alcohol which are renewable raw materials [28,29]. Owing to the presence of a hydrophilic end and a hydrophobic end, the product exhibits surface active properties, thus, its ability to reduce surface tension and hence increase the bioavailability of these hydrocarbons.

Several works are available in literature on the remediation of hydrocarbons using mostly bio stimulation and bioaugmentation approach. Bioavailability has however remained understudied [30, Harmsen and Naidu, 2015; [31,32]. Also, this work explores a novel application for Alkyl polyglucosides, as its use as surfactants in the bioremediation of hydrocarbons arising from crude oil contamination is yet to be fully explored.

## 2. MATERIALS AND METHODS

### 2.1 Description of Study Area

The study area were soil samples were collected are Ozuoba, Rukpokwu and Eneka communities in Obio-Akpor Local Government Area, Rivers State, Nigeria with the coordinates 7.06° longitude and 4.89° latitude. There is a the Trans Nigeria pipeline right of way (ROW) which is bounded to the north, east, south and west by a few residential buildings and farmlands. There are network of roads existing within the communities as presented in Fig. 1.

### 2.2 Sample Collection and Contamination with Crude Oil

This study was simulated in the laboratory using soil contaminated with crude oil samples.

Soil samples were collected from three different locations Ozuoba, Rukpokwu and Eneka all in Obio/Akpor Local government area of Rivers State Nigeria. The soil samples were air dried at room temperature, crushed, sieved with a 2 mm sieve and homogenized. The physico-chemical properties of the soil samples were determined as shown in Table 1.

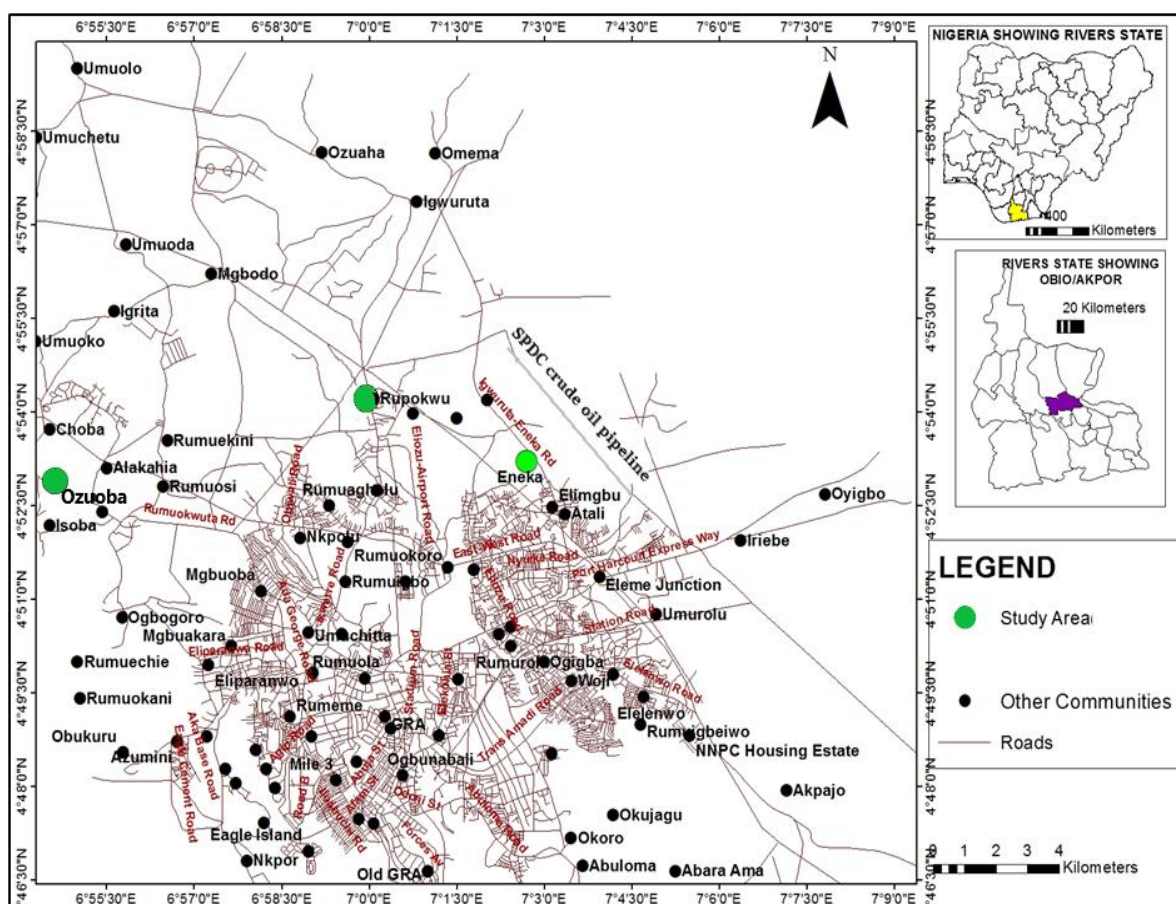


Fig. 1. Map of study area showing sample collection sites

Table 1. Physicochemical properties of the soil samples

Parameters	Eneka	Ozuoba	Rukpokwu
pH	6.997	6.725	6.683
EC ( $\mu\text{S}/\text{cm}$ )	94.52	53.68	72.7
Silt (%)	31%	60%	60%
Clay (%)	6%	1%	7%
Sand (%)	63%	39%	33%
Texture	Sandy loam	Silty Loam	Silty Loam
TPH (mg/kg)	278	66.8	62.8

Preparation of crude oil contaminated soil was carried out as follows. 250 g of each of the three soil samples was weighed and placed in polyethylene bags in duplicate. 20 mL of light crude (Rumuekpe) was uniformly spiked on one set of the samples and mixed together thoroughly. To the second set, 20 mL of heavy crude (Ogbain) was uniformly spiked and thoroughly mixed together also. These samples were set aside for 3 days. The TPH concentration of each of the set up was analyzed according to US EPA 8270 test method using Agilent Gas Liquid Chromatography.

### 2.3 Synthesis of Alkyl Polyglucoside Biosurfactant

The biosurfactant alkyl polyglycosides APG10 ( $\text{C}_6\text{H}_{11}\text{O}_5$ )<sub>n</sub>OR) used in this study was synthesized in the laboratory using the following chemicals; Glucose, Propanol, ParaToluene sulfonic acid (PTSA) and Decanol. All chemicals used in the synthesis were of analytical grade purchased from AccuStandard® obtained from Shanghai Fine Chemical Co., China. These chemicals were used in the synthesis of the Decyl glucoside (an Alkyl

polyglucoside). The chemicals were received and used without further purification.

The alkyl polyglucoside used in this work is Decyl glucoside. The alkyl polyglucoside was synthesized as described by El-Sukkary *et al.*, [29]. The decyl alkyl polyglucoside (APG10) was synthesized by an indirect method using paratoluene sulfonic acid (PTSA) as catalyst. In the first step, anhydrous glucose reacted with propyl alcohol. The second step included the trans acetalization of propyl glucoside intermediate with the decyl alcohol. In the initial step, of 5.4 g of anhydrous glucose was dissolved in 23 mL of propanol in the presence of 0.4 g PTSA and heated at 90°C for 1 hour. In this step propyl glucoside was formed. Then, 25 mL of decanol and 0.06g of PTSA were slowly added and the temperature increased to 110°C. The reaction was quenched by the addition of 1% alcoholic NaOH. The product was separated as water soluble sodium salt while the excess alcohol was recovered by distillation of the volatile ether solvent. Finally, yellowish liquid was obtained as the product. FT-IR analysis of the synthesized APG was carried out to confirm the product.

## 2.4 Remediation of the Contaminated Soil Samples with Alkyl Polyglucoside Bio-Surfactant

The contaminated soil samples were spiked with Decylglucoside and water and stirred. A total of 6

experimental set up was used (250 g of soil spiked with 20 mL of crude). 3 of the setup were spiked with light crude (one from each site), and another 3 were spiked with heavy crude. 10 mL of water was added to each of the soil setup in this batch, and stirred to mix homogeneously, and then 8 mL of the synthesized alkyl polyglucoside was added to each setup and stirred to ensure adequate mixing. This was left to stand for 30 days.

All the setup was stirred twice a week and additional 10 mL of water spiked on them after two weeks. The samples were then analyzed for TPH after 30 days.

## 3. RESULTS AND DISCUSSION

### 3.1 Soil Analysis

The soil samples used for this study from the results obtained (see Table 1) show that the soil samples are neutral as the 3 samples had a pH of approximately 7 each. Eneka soil sample from soil texture analysis was predominantly sand, while the other two (Ozuoba and Rukpokwu were predominantly silt. The TPH (mg/kg) concentration of these samples 278, 66.8 and 62.8 for Eneka, Ozuoba and Rukpokwu respectively.

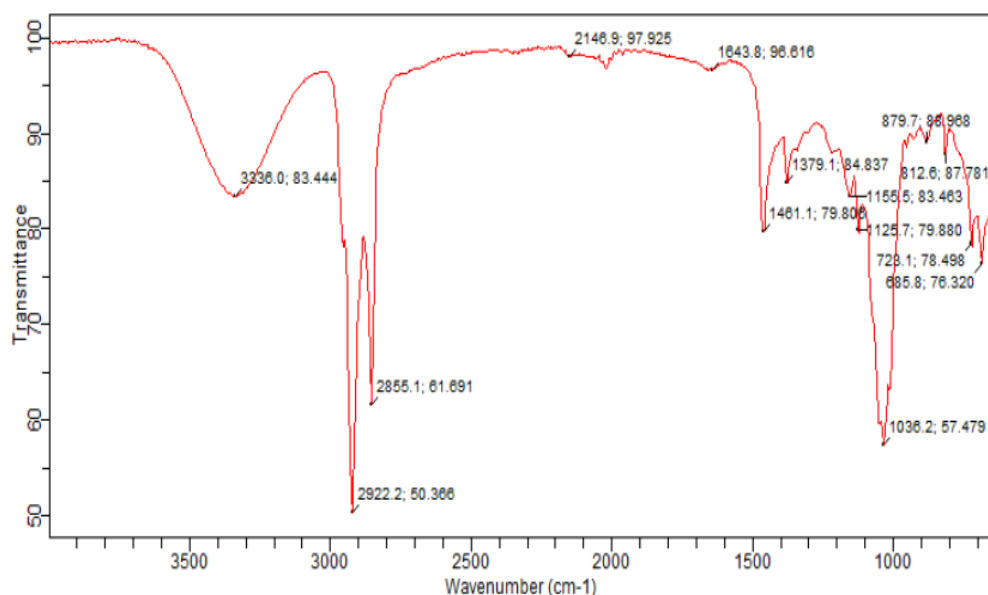


Fig. 2. FTIR of synthesized Alkyl polyglucoside (C<sub>10</sub> APG)

**Table 2. TPH concentration of soil samples before and after remediation with biosurfactant**

Soil sample	Before Remediation			After remediation		
	Eneka	Ozuoba	Rukpokwu	Eneka	Ozuoba	Rukpokwu
	<b>Medium crude oil</b>					
TPH (mg/kg)	15744.00	11359.00	11470.00	4276.00	4266.00	3205.00
% reduct.				72.84	62.44	72.05
	<b>Light crude oil</b>					
TPH (mg/kg)	11339.00	10662.00	10226.00	2981.00	3879.00	4245.00
% reduct.				73.71	63.62	58.49
<b>TPH (mg/kg)</b>	<b>Light crude oil</b>					
<b>CONTROL SAMPLES</b>	11290.00	10540.00	10350.00	10900.00	10250.00	10128.00
% reduct.				3.45	2.75	2.14

### 3.2 Characterization of Synthesized Alkyl Polyglucoside Bio-surfactant

FTIR spectroscopy was used to confirm the synthesized product. Fig. 2 shows the spectrum of the product while Table 3 is a list of the functional groups present in the product. The groups present are similar to that from literature [29,33,34]. The characteristic ether linkage which is usually a weak band in the range 1120-1170cm<sup>-1</sup>, observed at 1155.5cm<sup>-1</sup>, and the C–O band observed at 1036.2cm<sup>-1</sup> was used to confirm the synthesised product as an alkyl polyglucoside.

### 3.3 Results of Crude oil Contamination Soil Samples

The TPH content of the soil samples pre and post-contamination of soil samples are shown in Tables 1 and 2. The soil samples showed higher concentration of hydrocarbon when contaminated with medium crude and slightly lower concentration with light crude. Medium crude oil contain a higher percentage of hydrocarbons with higher molecular weight, this is responsible for the larger concentration of contaminants in the soil samples contaminated with medium crude compared to that with light crude. The result also shows that the two soil samples (Ozuoba and Rukpokwu) that are silty loam retained more contaminants when compared to that retained by Eneka soil sample which is predominantly sand. The bioavailability of petroleum hydrocarbon compounds in contaminated soil was an essential factor that

influenced the effectiveness of the remediation process with biosurfactants (Liu et al., 2016). Petroleum hydrocarbons tended to be strongly absorbed to soil organic matter because of their hydrophobicity and low solubility, and their low release from the soil matrix to the aqueous phase reduced their availability to microorganisms for degradation. In many studies, surfactants were applied to overcome this limitation [35].

The efficiency of the surfactant (Alkyl polyglucoside) at degrading total petroleum hydrocarbons was shown by the reduction in TPH concentration. It was observed that there was a rapid increase in the biodegradation rates of TPH during 30 days of in all treatments of the soil contaminated samples used in the study. The result was in line with report of Li et al. [36], who stipulated that the biodegradation of TPH was greatly enhanced because due to the presence of Alkyl polyglucoside in soil samples.

The percentage efficiency of Alkyl polyglucoside in the remediation of petroleum hydrocarbons after 30 days was calculated using the following equation 1.

$$\begin{aligned} \text{Efficiency (\%)} &= \\ & \frac{\text{Decrease in TPH concentration}}{\text{Initial concentration}} \times 100 \\ &= \frac{C_{(\text{initial})} - C_{(\text{final})}}{C_{(\text{initial})}} \times 100 \quad (1) \end{aligned}$$

Where:

$C_{(initial)}$  = Initial concentration of TPH before remediation.

$C_{(final)}$  = Final concentration of TPH after remediation.

The high efficiency shown can be attributed to the effect of the bio-surfactant; it increases the solubility of hydrocarbons making it more accessible for microorganisms, as their low water solubility limits their availability to microorganisms [23].

Table 2 shows that samples contaminated with medium crude, the initial TPH content for Eneka, Ozuoba and Rukpokwu were 15744 mg/kg, 11359 mg/kg and 11470 mg/kg respectively.

These values reduced to 4276 mg/kg, 4265 mg/kg, and 3205 mg/kg after remediation, a reduction of 72.84%, 62.44% and 72.05% respectively. Soil samples remediated light crude contaminated samples also showed similar reduction in the TPH content having reduced from 11339mg/kg to 2981 mg/kg, 10662mg/kg to 3879 mg/kg, and 10226mg/kg to 4245 mg/kg respectively, a reduction of 73.71%, 63.62 % and 58.49% respectively. While the control samples showed TPH of 11290.00 mg/kg, 10540.00 mg/kg and 10350 mg/kg respectively. After thirty days the TPH values of control samples were 10900 mg/kg, 10250 mg/kg, 10128 mg/kg respectively. This showed a percentage reduction of 3.45%, 2.75% and 2.14 % respectively.

The comparison of the level of reduction of the remediated soil samples are shown in Figs. 4

and 5. The highest reduction was recorded for Eneka soil sample contaminated by light crude, which showed a decrease of approximately 74%, this can be attributed to the ease with which the light molecular weight hydrocarbon undergoes volatilisation and evaporation in sand [37,38].

### 3.4 Remediation of Contaminated Soil Samples with Alkyl Polyglucoside

The results of remediation of Total Petroleum contaminated (TPH) soil samples before and after remediation for light and medium crude oil are presented in Table 2. The result shows clearly a large decrease in the TPH concentration at the end of thirty (30) days. According to Atlas and Bartha, 1992, the efficacy of a remediation process can be directly measured by monitoring the rate of disappearance of hydrocarbons. The result in this study suggests a rapid degradation and disappearance of petroleum hydrocarbon.

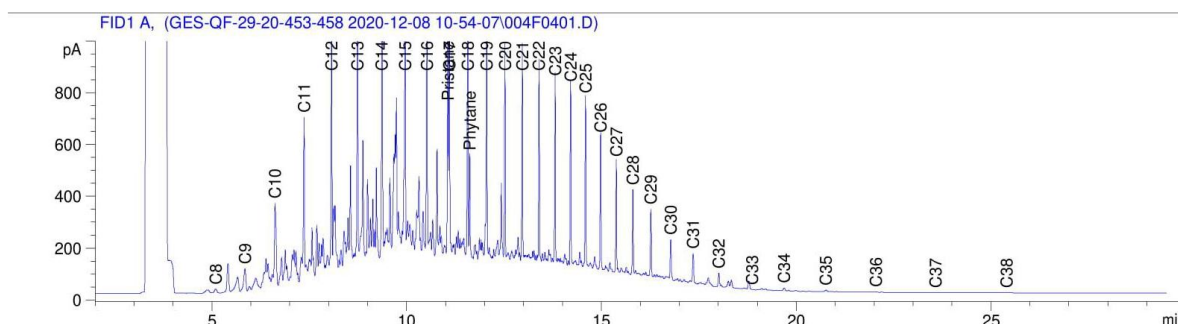
The Effects of Alkyl polyglucoside on the removal of TPH fractions on the different fractions of petroleum hydrocarbons from treatments of the various samples are shown by the disappearance of the petroleum hydrocarbons as seen in the chromatograms (Figs. 3a-e), while Fig. 3c is the chromatogram of the control soil sample from the same geographical study area, contaminated with crude oil over the same period of time, without biosurfactant-assisted remediation. There was a slight percentage reduction in TPH level for all contaminated samples without bio-surfactant as shown in Table 2. This may be attributed to either natural attenuation or evaporation of the light ends of the hydrocarbons [39]. The lower hydrocarbon chains present in soil samples are volatile and

**Table 3. The FTIR spectrum values and functional groups of Alkyl polyglucoside bio-surfactant**

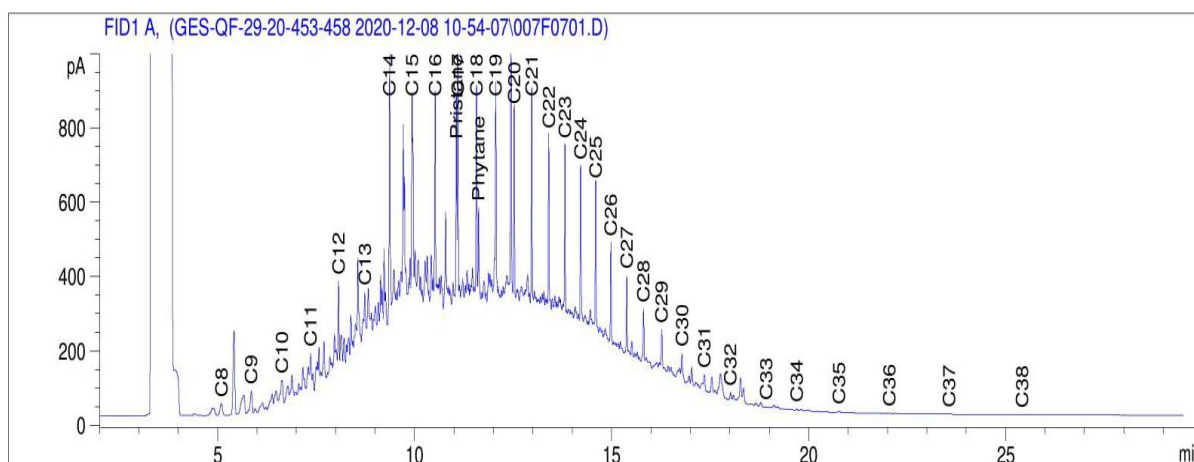
Functional Group	Wave no. (cm <sup>-1</sup> )
<b>CH<sub>2</sub></b>	
Multiple (CH <sub>2</sub> ) rock	728.10
C - H Asymmetric bending	1461.10
C-H Symmetric bending	1379.10
C-H Symmetric stretch	2856.10
<b>CH<sub>3</sub></b>	
C-H Symmetric bending	1379.10
C-H Asymmetric bending	1643.80
C-H Symmetric stretch	2922.20
C-H Asymmetric stretch	2856.10
O-H	3336.00
C -O	1036.20
C-O-C	1155.50

easily biodegraded in soils. It is also a well known fact that shorter chain hydrocarbons (< C10) are easily degraded as they have lower molecular weight, while medium - length hydrocarbons (C10-C16) and long-chain

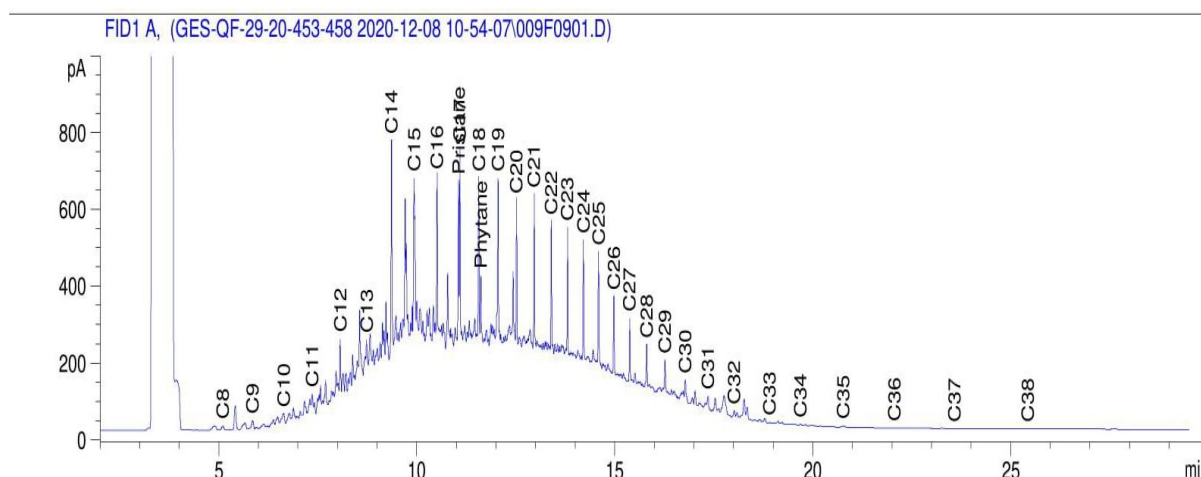
hydrocarbons (> C16) are degraded at a relatively slow rate as due to their greater hydrophobicity that causes low bioavailability to microorganisms [40].



**Fig. 3a. Representative chromatogram of TPH for light crude oil post contaminated samples**



**Fig. 3b. Representative chromatogram of TPH for medium crude oil post contaminated samples**



**Fig. 3c. Representative chromatogram of TPH for control samples**



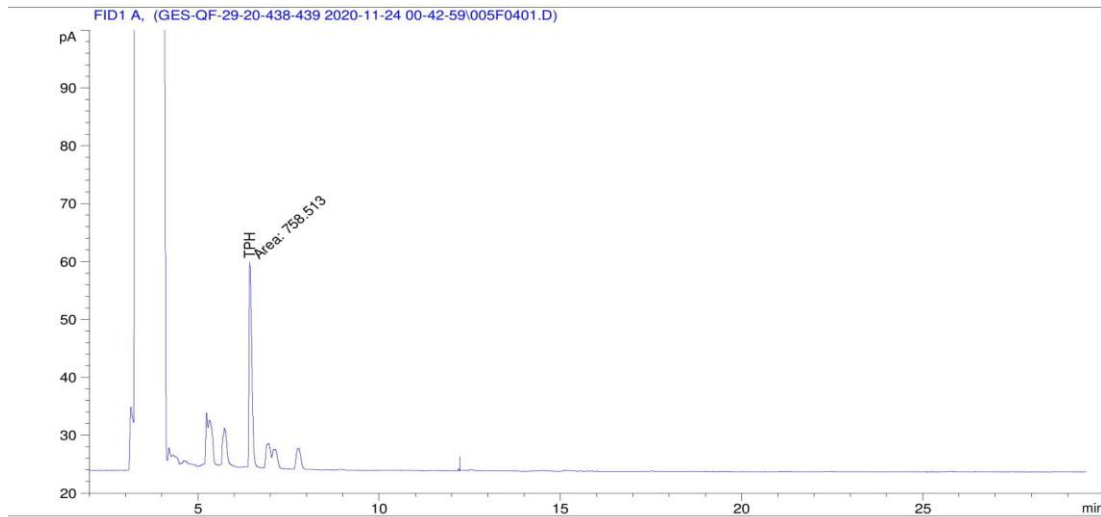


Fig. 3d. Representative chromatogram of TPH for medium crude oil samples after remediation

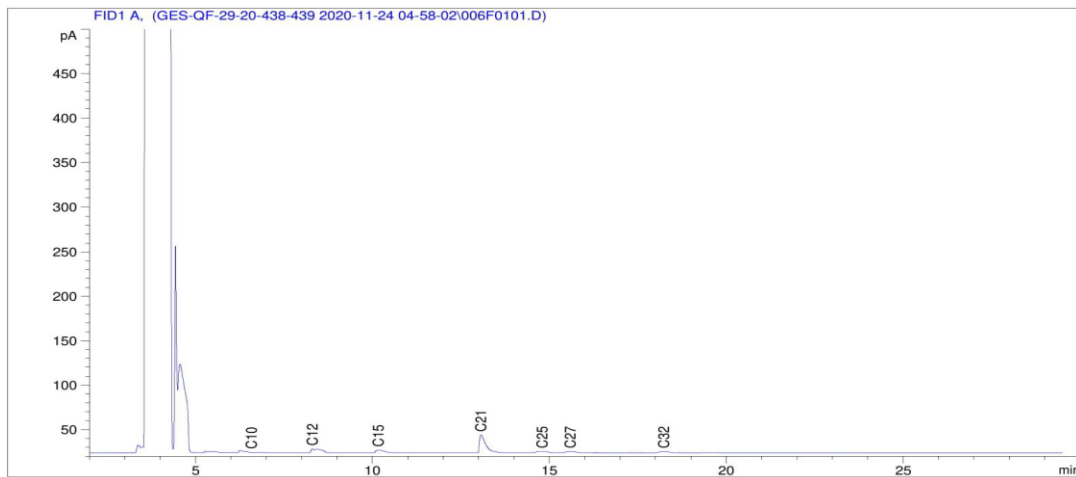


Fig. 3e. Representative chromatogram of TPH for light crude oil samples after remediation

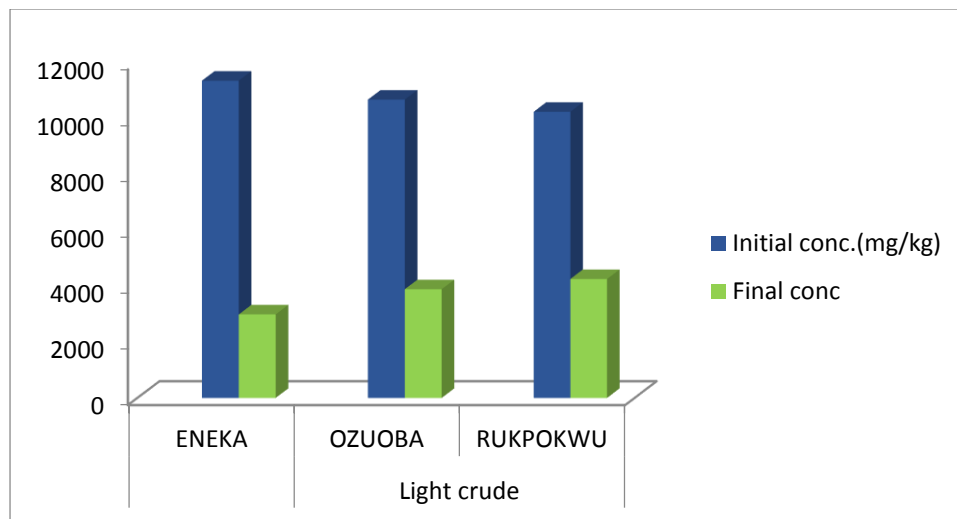
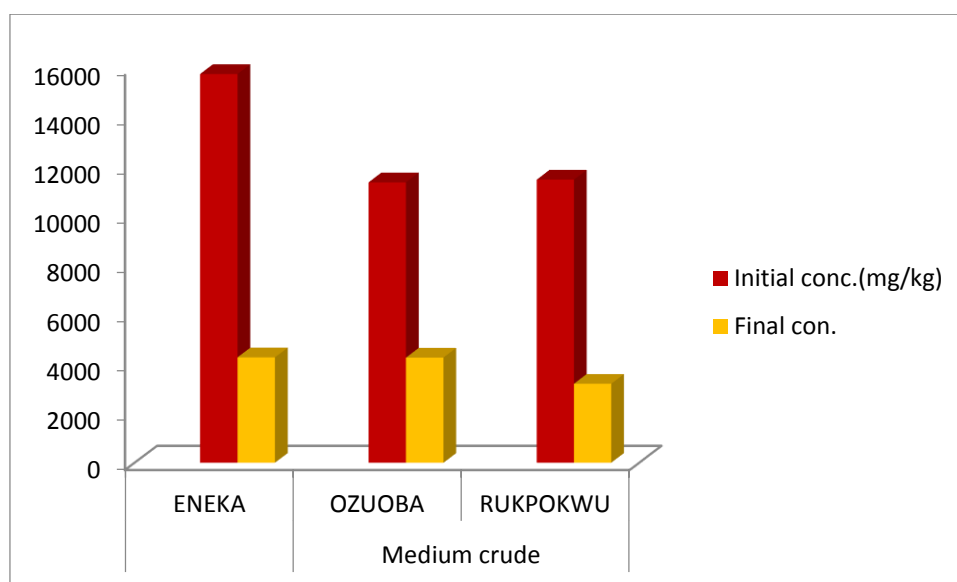


Fig. 4. Bar chart showing reduction in the concentration of TPH for light crude oil samples



**Fig. 5. Bar chart showing reduction in the concentration of TPH for medium crude oil samples**

#### 4. CONCLUSION

Petroleum hydrocarbon is known to contain contaminants toxic to the environment. These contaminants are also not readily degradable due to their hydrophobic nature and low solubility hence hindering accessibility to degrading microorganisms. This research showed the result of synthesised biosurfactant experiments on the biodegradation of petroleum hydrocarbons. Results obtained in this study showed that the bioremediation of petroleum hydrocarbon contaminated soil can be enhanced by treating the contaminated soil with alkyl polyglucoside. This bio-friendly surfactant has the ability to increase the solubility of the hydrocarbons making them more susceptible and easily accessible to the microorganisms for degradation, thus increasing the efficiency of the remediation process. Being eco-friendly, it also has the advantage of being completely degradable and has no toxic or unwanted intermediate or by product. Molecular parameters show that removal of petroleum hydrocarbons occurs during biodegradation of the crude oil, in addition to alteration of other petroleum hydrocarbons. It was observed that the low molecular weight n-alkanes were lost in all the samples and the highest percentage reduction was recorded for soil samples contaminated with light crude, which showed a decrease of approximately 74%, this can be attributed to the ease with which the light molecular weight hydrocarbon undergoes volatilisation and evaporation in sand.

#### DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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