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Biodegradation Potential of Bacteria Isolated from Crude Oil Polluted Site in South South, Nigeria

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Authors' contributions

This work was carried out by the collaborative efforts of all authors. Authors AAI and SAW designed the study. Author AAI wrote the first manuscript and carried out all laboratory work described in the study. Authors RRN and SAW read and approved the final manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study aimed to determine the biodegradative potential of crude oil by *Chryseobacterium sp.* and *Lysinibacillus fusiformis* isolated from Crude Oil site in Gokana LGA, of Rivers State.
Study Design: Research was designed to evaluate the speed of degradation of crude oil by bacterial species over a period of 28 days.
Place and Duration of Study: Study was carried out in the Microbiology Laboratory of Rivers State University from March to April 2018.
Methodology: Four experimental set ups were used: mineral salt broth with crude oil only (Control), *Chryseobacterium sp.* in mineral salt culture with crude oil (Set-up 1), *Lysinibacillus fusiformis* in mineral salt culture with crude oil (Set-up 2) and *Chryseobacterium sp.+Lysinibacillus fusiformis* in mineral salt culture with crude oil (Set-up 3). Analysis of pH and Total Viable Counts was carried out at weekly intervals while Total Petroleum Hydrocarbon was carried out at bi-weekly intervals.
Results: Results showed a steady decline in pH from almost neutral (6.1) to acidic with mixed culture set up having the lowest value of 4.19 < *Lysinibacillus fusiformis* set up of 4.22 < *Chryseobacterium sp.* set up of 4.34. Total Petroleum Hydrocarbon followed the same trend of

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steady decline from 74.8 mg/L with *Lysinibacillus fusiformis* species having the least value of 40.44 mg/L < mixed culture of 44.85 mg/L < *Chryseobacterium sp.* of 51.08 mg/L. Total Viable Counts (Log10 cfu/mL) of bacteria showed increase in growth in all set ups. *Lysinibacillus fusiformis* increased from 5.10±0.01 to 6.85±0.05 > the consortium increased from 5.21±0.01 to 6.84±0.04 > *Chryseobacterium sp.* increased from 5.30±0.01 to 6.73±0.06 from beginning to end of the experiment.

Conclusion: Results showed that *Lysinibacillus fusiformis* had greater capacity to degrade crude oil, followed by the mixed culture and lastly, *Chryseobacterium sp.* These bacterial species can be harnessed and used for clean-up of crude oil contaminated environment.

Keywords: Biodegradation; Chryseobacterium sp.; Lysinibacillus fusiformis; mixed culture; mineral salt broth.

1. INTRODUCTION

Crude Oil is a naturally occurring complex mixture of hydrocarbon and non-hydrocarbon compounds such as Sulphur, Nitrogen and Oxygen; which at appropriate concentrations possess toxicity towards living systems [1]. Biodegradation is the disintegration of complex chemicals through a biological process. This term is used to describe three major types of changes in a molecule; (a) a minor change in the functional groups attached to an organic compound, such as substitution of a hydroxyl group for a chlorine group; (b) an actual breaking of the organic compound into organic fragments in a manner that the original compound can be restructured and (c) the complete degradation of an organic compound to minerals that can be recycled [2]. Bacteria are a class of microorganisms known to degrade petroleum hydrocarbons from contaminated environments and use these compounds as sole carbon and energy sources. The extent of degradation process is dependent on the genetic make-up of the microorganism to introduce molecular oxygen in the hydrocarbon and to generate the intermediates that enter the general energy-yielding metabolic pathway of the cell [3]. Usually, degradation takes place in the presence of oxygen via aerobic respiration. The hydrocarbon loses electrons and is oxidized while oxygen gains electrons and is reduced; resulting in carbon dioxide and water as final products [4]. Bacteria that are known to degrade petroleum products include species of *Pseudomonas, Aeromonas, Moraxella, Flavobacterium, Nocardia, Bacillus, Sphingomonas, Micrococcus* and so on. [5]. In the world over; scientists, environmentalists and conservationists are posed with the challenge on how to upturn the toxic effects of crude oil contamination in soil, air and water [6]. The fractional distillation of crude oil to manufacture several products as well as indiscriminate usage, improper disposal and accidental leakage from pipes has led to the contamination of land, air and water ecosystems. Soil pollution is of great importance in the scheme of biotic and abiotic activities; consequently, leading to the bio-magnification of these toxic components up to the food chain and into the human populace [7]. In soils, crude oil is absorbed into the soil matrix and vaporous compounds will eventually partition to the atmosphere; with time hydrocarbons that are available for the microbial attack will be slowly degraded [8]. The Niger-Delta region of Nigeria known for its thick rainforests, wetlands, waterways and creeks is home to large deposits of crude oil; although oil exploitation activities started in 1958, parts of this region have not fully enjoyed the benefits of the exploitation and exploration activities of this all-important natural resource [9]. With a population size of about 27 million covering an area of 70,000 square kilometres, 5,000 communities, 50 ethnic groups and 250 dialects; this region is a constant reminder of the effects of poor oil exploration practices, pipe leakage and vandalization [9]. Today, inhabitants of most of these communities have lost their sources of livelihood (farmlands used for animal husbandry and crop cultivation; natural ponds used in fish farming), no potable drinking water; leading to general youth restiveness. Crude oil pollution has over the years become a problem in the Niger Delta region of Nigeria, affecting soil ecosystems and rendering acres of viable land unfit for any form of use. Hence, it has become imperative that effective hydrocarbon degraders be isolated from the polluted soil and used for degradation of these mutagenic compounds in the field.

2. MATERIALS AND METHODS

2.1 Study Area

Soil samples were collected from the study site within coordinates 4.30.03N & 7.13.46E 19km

and 4.28.45N & 7.14.42E 19 km in Kegbara dere Creek in Gokana Local Government Area of Rivers State.

2.2 Sample Collection

Soil samples were collected by adopting the Food and Agriculture Organisation [10] guideline, using a sterile soil auger to make a depth of 0-5 cm of topsoil. The soil samples for analysis were collected into fresh unused black polythene bags perforated for aeration. The samples were transported within 2 hours of collection to the Microbiology Laboratory of the Rivers State University.

2.3 Isolation and Identification of Bacterial Species

Two bacterial species used for this study were isolated from the top 5cm of homogenously mixed and sieved crude oil polluted soil from Kegbara dere Creek in Gokana Local Government Area of Rivers State. One gram (1.0g) of soil sample was weighed and introduced into sterile normal saline (8.5g of NaCl in 1000ml of distilled water) under aseptic conditions. Ten-fold serial dilution was carried out up to 10^{-5} and 10^{-6} dilutions. Afterwards, a 0.1ml aliquot of each dilution was spread plated onto sterile solidified mineral salt agar using the vapour transfer method in duplicates. Two distinct colonies were picked and cultured on nutrient agar and blood agar media respectively. The plates were incubated at 37^0C for 24hours. Distinct colonies were counted to obtain the colony forming unit per gram (CFU g^{-1}) of the soil sample [11].

Thereafter, two distinct colonies with varying cultural characteristics, suspected to be *Chryseobacterium sp.* and *Lysinibacillus fusiformis*, were picked and sub-cultured onto freshly prepared nutrient agar plates which were incubated at 37^0C for 24 hours. Biochemical tests were carried out as illustrated by Cheesebrough [12]. The purified colonies were stored in 10% glycerol solution at freezing temperature to serve as stock culture for further identification. The purified isolates were taken for molecular characterization and identification to strain level.

2.4 Biodegradation Test Using Two Bacterial Species

The two species of bacteria (*Chryseobacterium sp. and Lysinibacillus fusiformis*) isolated from the soil samples were used for the biodegradation set up. Each set up had Mineral Salt Broth containing 1000ml distilled water, 0.5g K_2HPO_4, 0.3g $MgSO_4.7H_2O$, 0.3g NaCl, 0.2g $MnSO_4.H_2O$, 0.02g $FeSO_4$. $6H_2O$, 0.03g $NaNO_3$, 0.3g ZnCl; all measured into four 1000ml conical flasks and autoclaved; with 5ml of sterilized crude oil been aseptically added into each flask and plugged with cotton wool. For the single bacteria set up, serial dilutions from nutrient broth cultures were made and aliquots plated on nutrient agar using the spread plate method to determine the number of bacterial cells in each aliquot. It was within the range of 30-300 cells. Consequently, 1ml from 10^{-4} dilution was aseptically added into each setup; while 0.5ml of each organism was added in the consortium set up. Each flask was agitated using a rotatory shaker for 30 minutes daily to allow for aeration and even distribution of nutrients and the pollutant. During the incubation period, pH readings were taken with a pH meter in duplicates; while total viable counts were monitored in triplicates using the spread plate method on nutrient agar. Total Petroleum Hydrocarbon readings were taken using Gas Chromatography method with Flame Ionisation Detector (Agilent HP 5890 series II) [13]. Total Viable Counts and pH readings were monitored on a 7-day basis (day 1, 7, 14, 21, 28) while the Total Petroleum Hydrocarbon readings were taken on a 14-day basis (day 1, 14, 28).

3. RESULTS AND DISCUSSION

The two bacterial species (*Chryseobacterium sp. and Lysinibacillus fusiformis*) were used for this study after they showed growth on the mineral salt medium. Results of this study showed that under suitable temperature, oxygen and enough nutrients; biodegradation rate increased in each setup. Hydrogen ion concentration has been known to affect the rate of degradation by controlling the speed of enzyme-mediated reactions over a wide range, but optimally from 6.5 to 8.5 [14]. During this experiment, the pH values of each set up decreased progressively from almost neutral to very acidic level due to the breakdown of the petroleum hydrocarbons to CO_2 and H_2O; and increased growth of bacterial cells was observed, with the mixed culture being the lowest at 4.19 from an initial value of 6.1.

While there was increased biomass accumulation with the help of oxygenases acting on hydrocarbon components, the cycle of biodegradation continues if all factors affecting microbial degradation are kept constant. Fig. 2

shows a steady increase in bacterial growth as fast as it was able to utilize the hydrocarbon components *Chryseobacterium sp.* evidently could not utilize the hydrocarbon components at a similar rate as the two other set ups, despite its significantly high growth over time, with a growth range of 5.30±0.01Log10cfu/mL to 6.73±0.06 Log10cfu/ml. Although it is suspected that due to competition between *Chryseobacterium sp.* and *Bacillus* species in the mixed-culture set up with growth range of 5.21±0.01Log10cfu/mL to 6.84±0.04Log10cfu/mL and due to cell death probably occurred at some point, the growth rate was not as high as that of *Lysinibacillus fusiformis* with a growth range of 5.10±0.0105Log10cfu/mL to 6.85±0.05Log10 cfu/ml.

As corroborated by [15], due to the high growth rate of *Lysinibacillus fusiformis* throughout the experiment, the set up showed to be the best degrader of petroleum hydrocarbon with a value of 40.44 mg/L. The mixed culture followed with a value of 44.85 mg/L and *Chryseobacterium sp.* with a highest value of 51.08 mg/L been the least degrader. Fig. 3 shows the summary of the gradual progression of how the petroleum hydrocarbon was degraded over time.

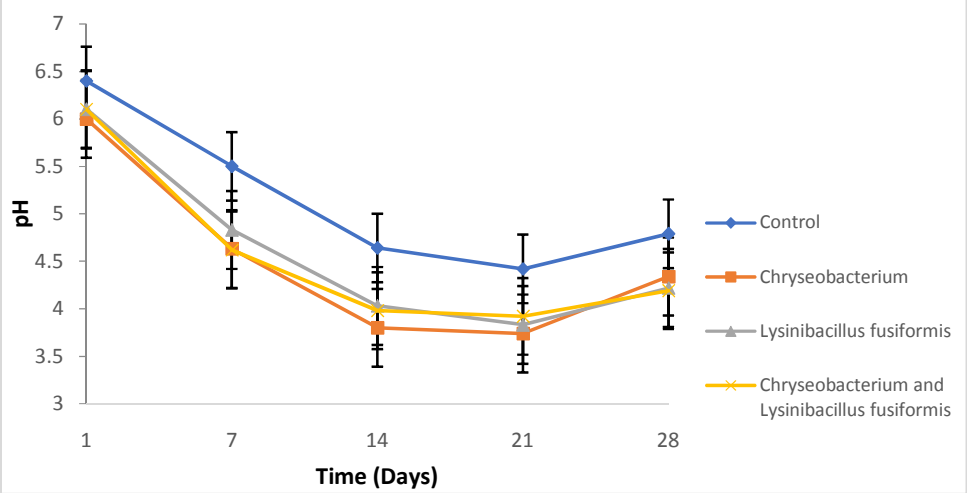


Fig. 1. Hydrogen ion concentration (pH) during biodegradation of crude oil polluted aquatic ecosystem using mineral salt broth

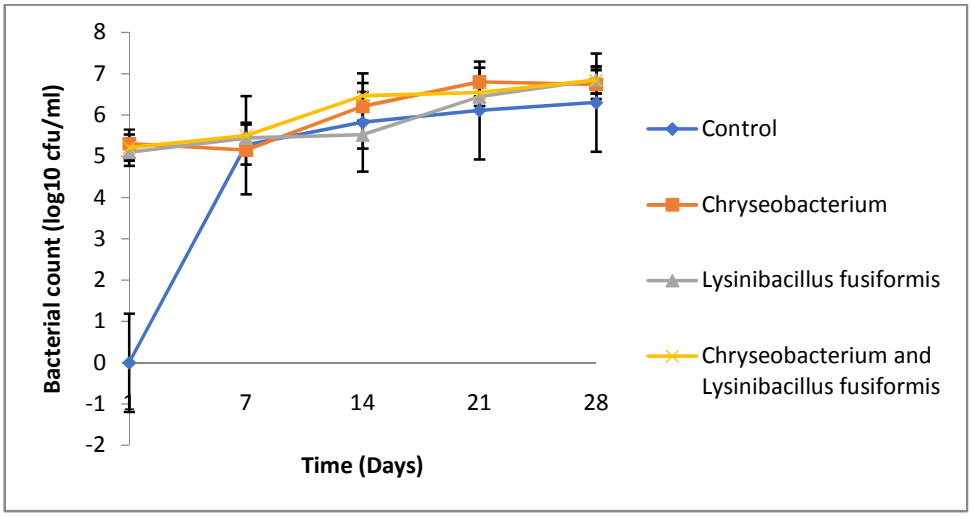


Fig. 2. Petroleum Utilizing Bacteria (log10 cfu/ml) during biodegradation of crude oil polluted aquatic ecosystem using mineral salt broth

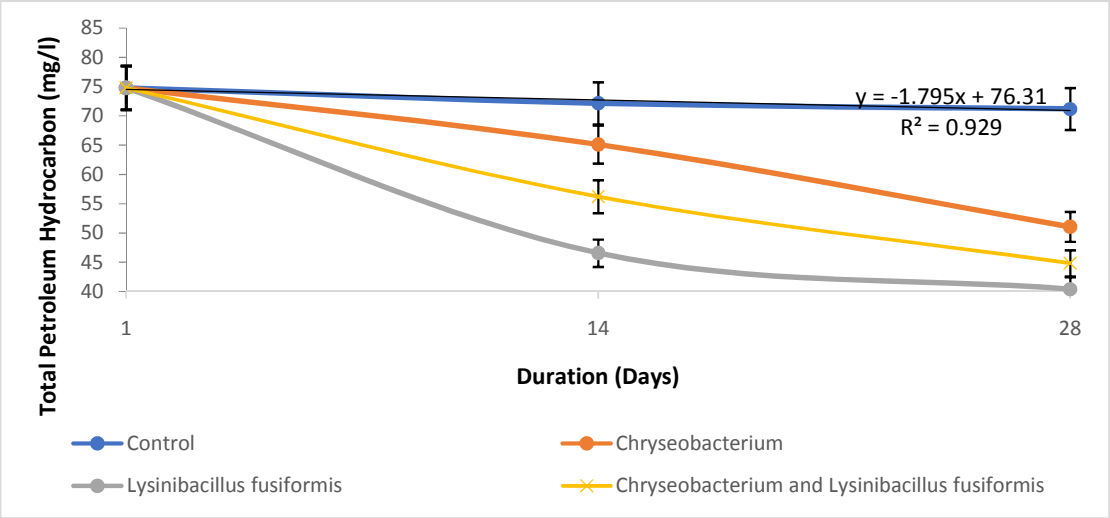


Fig. 3. Total Petroleum Hydrocarbon during biodegradation of crude oil polluted aquatic ecosystem using mineral salt broth

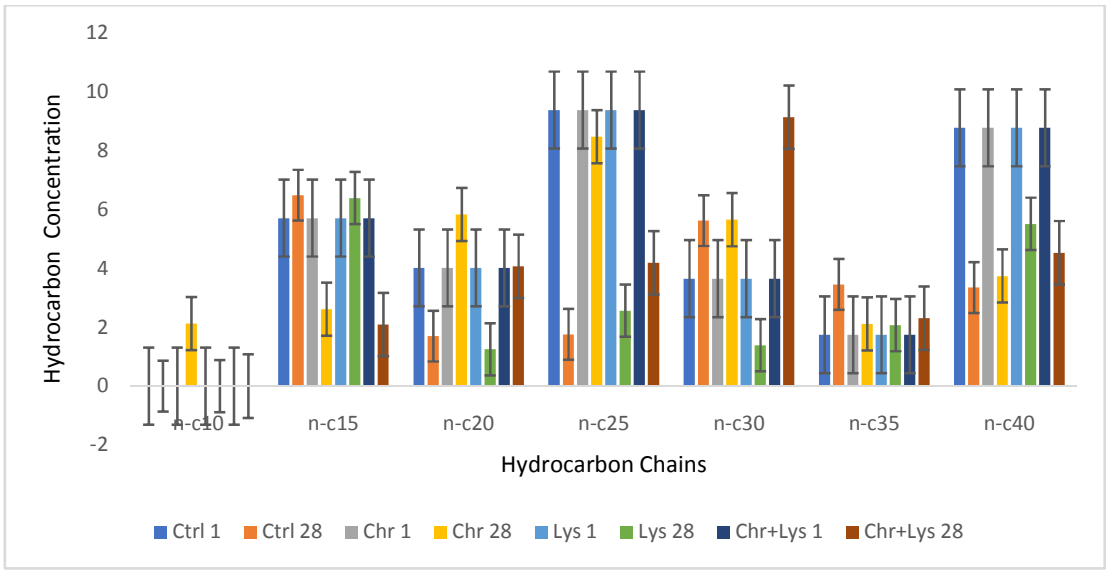


Fig. 4. Hydrocarbon chains degradation comparison between Day 1 and Day 28

Fig. 4 shows the comparison between Day 1 and Day 28 of hydrocarbon chains; c10, c15, c20, c25, c30, c35 and c40; for each of the set-ups. Chain c15 was degraded the highest by Set-up after 28 days of incubation. Chains c20, c30 and c35 were degraded the most by Set-up 2 [16].

Figs. 5-12 shows the peaks and values of petroleum hydrocarbons and how the chains degraded over time.

Table 1 shows comparison between Days 1, 14 and 28 on each of the hydrocarbon chains. Chain c11 was degraded to 0.003ppm on Day 28 from an initial amount of 0.355ppm on Day 1 by Set-up 1 and 2 respectively. Chain c12 was significantly reduced from initial of 0.089ppm to 0.005ppm, 0.0005ppm and 0.0002ppm by Set-up 1, 2 and 3 respectively. Chain c17 was reduced from 0.691ppm to 0.007ppm by Set-up 2 at Day 28. Chain c19 was greatly reduced from an initial

Table 1. Comparative analysis of hydrocarbon chains over 28 days incubation period

	Day 1	Day 14	Day 28		Day 1	Day 14	Day 28		Day 1	Day 14	Day 28
n-c8				**Phytane**				**n-c30**			
Ctrl	0	0	0	***Ctrl***	1.983	7.087	8.514	***Ctrl***	0.365	4.094	0.056
Chr	0	0	0	***Chr***	1.983	6.421	2.968	***Chr***	0.365	0.653	5.652
Lys	0	0	0	***Lys***	1.983	4.642	2.346	***Lys***	0.365	0.441	1.389
Chr+Lys	0	0.011	0	***Chr+Lys***	1.983	4.369	2.353	***Chr+Lys***	0.365	0.246	0.913
n-c9				**n-c19**				**n-c31**			
Ctrl	0	0	0	***Ctrl***	27.251	0.225	1.278	***Ctrl***	1.440	4.000	1.195
Chr	0	0	0	***Chr***	27.251	2.638	0.046	***Chr***	1.440	0.408	5.556
Lys	0	0	0	***Lys***	27.251	2.112	0.017	***Lys***	1.440	0.328	1.481
Chr+Lys	0	0.011	0	***Chr+Lys***	27.251	0.099	0.035	***Chr+Lys***	1.440	0.490	0.182
n-c10				**n-c20**				**n-c32**			
Ctrl	0	0.016	0	***Ctrl***	0.401	0.297	1.700	***Ctrl***	0.279	0.016	0.124
Chr	0	0.010	0.021	***Chr***	0.401	0.198	0.583	***Chr***	0.279	0.228	0.016
Lys	0	0.00003	0	***Lys***	0.401	0.018	0.125	***Lys***	0.279	0.150	0.007
Chr+Lys	0	0.010	0	***Chr+Lys***	0.401	0.074	0.407	***Chr+Lys***	0.279	0.411	0.011
n-c11				**n-c21**				**n-c33**			
Ctrl	0.355	0.013	0.043	***Ctrl***	1.211	0.133	0.513	***Ctrl***	2.539	2.198	0.401
Chr	0.355	0.003	0.003	***Chr***	1.211	0.640	0.044	***Chr***	2.539	0.905	4.279
Lys	0.355	0.006	0.003	***Lys***	1.211	0.519	0.007	***Lys***	2.539	0.594	1.119
Chr+Lys	0.355	0.012	0	***Chr+Lys***	1.211	0.355	1.751	***Chr+Lys***	2.539	0.500	0.932
n-c12				**n-c22**				**n-c34**			
Ctrl	0.089	0.004	0.092	***Ctrl***	0.085	0.033	0.073	***Ctrl***	2.149	1.196	0.055
Chr	0.089	0.430	0.005	***Chr***	0.085	0.091	0.041	***Chr***	2.149	0.278	2.015
Lys	0.089	0.086	0.0005	***Lys***	0.085	0.088	0.055	***Lys***	2.149	0.022	0.559
Chr+Lys	0.089	0.025	0.0002	***Chr+Lys***	0.085	0.765	0.195	***Chr+Lys***	2.149	0.575	0.509
n-c13				**n-c23**				**n-c35**			
Ctrl	0.083	2.568	0.469	***Ctrl***	0.254	0.677	0.252	***Ctrl***	1.744	0.004	0.035
Chr	0.083	1.125	0.481	***Chr***	0.254	0.359	1.315	***Chr***	1.744	0.163	0.021
Lys	0.083	0.331	0.547	***Lys***	0.254	0.377	0.017	***Lys***	1.744	0.216	0.002
Chr+Lys	0.083	0.269	0.281	***Chr+Lys***	0.254	0.162	1.141	***Chr+Lys***	1.744	0.759	0.023

	Day 1	Day 14	Day 28		Day 1	Day 14	Day 28		Day 1	Day 14	Day 28
n-c14				**n-c24**				**n-c36**			
Ctrl	0.089	2.995	11.478	***Ctrl***	1.245	0.628	2.148	***Ctrl***	1.571	0.303	0.555
Chr	0.089	9.645	2.216	***Chr***	1.245	0.311	0.997	***Chr***	1.571	0.404	0.006
Lys	0.089	4.659	2.066	***Lys***	1.245	0.309	0.368	***Lys***	1.571	0.009	0.043
Chr+Lys	0.089	4.780	1.758	***Chr+Lys***	1.245	0.053	0.356	***Chr+Lys***	1.571	1.086	0.005
n-c15				**n-c25**				**n-c37**			
Ctrl	0.057	1.578	6.483	***Ctrl***	0.938	0.499	1.757	***Ctrl***	5.851	0.007	0.107
Chr	0.057	2.345	2.617	***Chr***	0.938	0.611	0.847	***Chr***	5.851	0.128	0.020
Lys	0.057	1.409	0.639	***Lys***	0.938	0.554	0.256	***Lys***	5.851	0.259	0.427
Chr+Lys	0.057	1.748	2.094	***Chr+Lys***	0.938	0.062	0.042	***Chr+Lys***	5.851	1.466	0.008
n-c16				**n-c26**				**n-c38**			
Ctrl	0.162	0.369	0.738	***Ctrl***	0.027	0.007	1.306	***Ctrl***	3.131	0.025	0.031
Chr	0.162	1.175	0.205	***Chr***	0.027	0.375	0.929	***Chr***	3.131	0.036	1.233
Lys	0.162	0.126	1.767	***Lys***	0.027	0.245	2.167	***Lys***	3.131	0.589	0.229
Chr+Lys	0.162	0.104	0.149	***Chr+Lys***	0.027	0.117	0.678	***Chr+Lys***	3.131	1.982	0.002
n-c17				**n-c27**				**n-c39**			
Ctrl	0.691	14.591	4.171	***Ctrl***	2.557	0.178	0.789	***Ctrl***	3.745	0.004	0.008
Chr	0.691	3.498	0.801	***Chr***	2.557	0.069	0.292	***Chr***	3.745	0.138	0.599
Lys	0.691	2.978	0.007	***Lys***	2.557	0.033	0.046	***Lys***	3.745	0.036	0.039
Chr+Lys	0.691	2.299	0.525	***Chr+Lys***	2.557	0.115	0.089	***Chr+Lys***	3.745	2.844	0.009

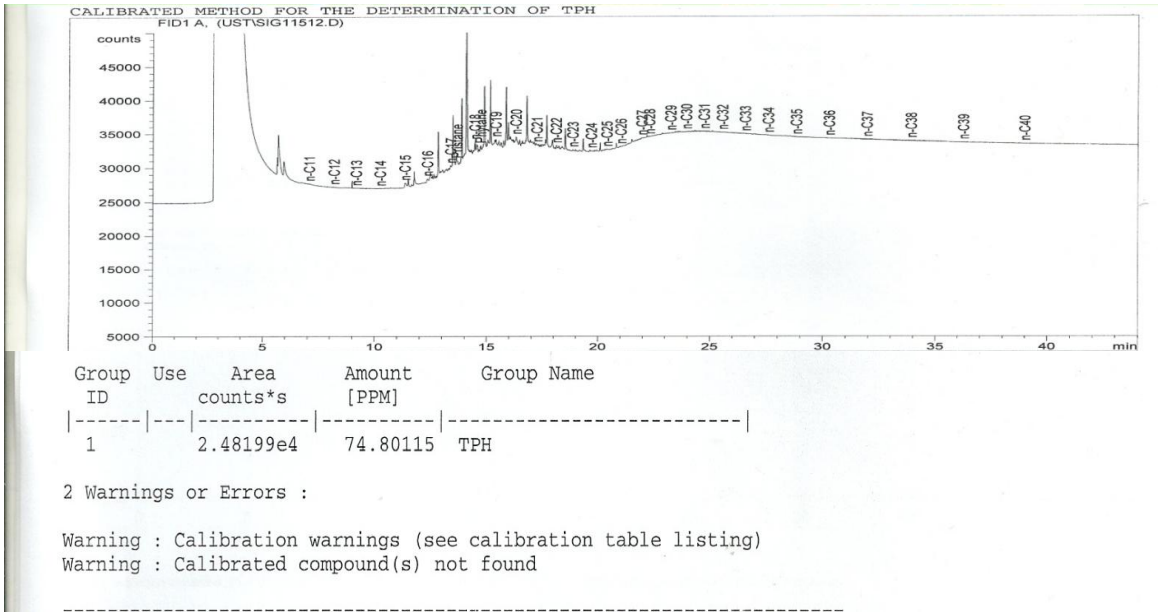


Fig. 5. Total petroleum hydrocarbon value and peaks on day 1 of crude oil polluted aquatic ecosystem using mineral salt broth

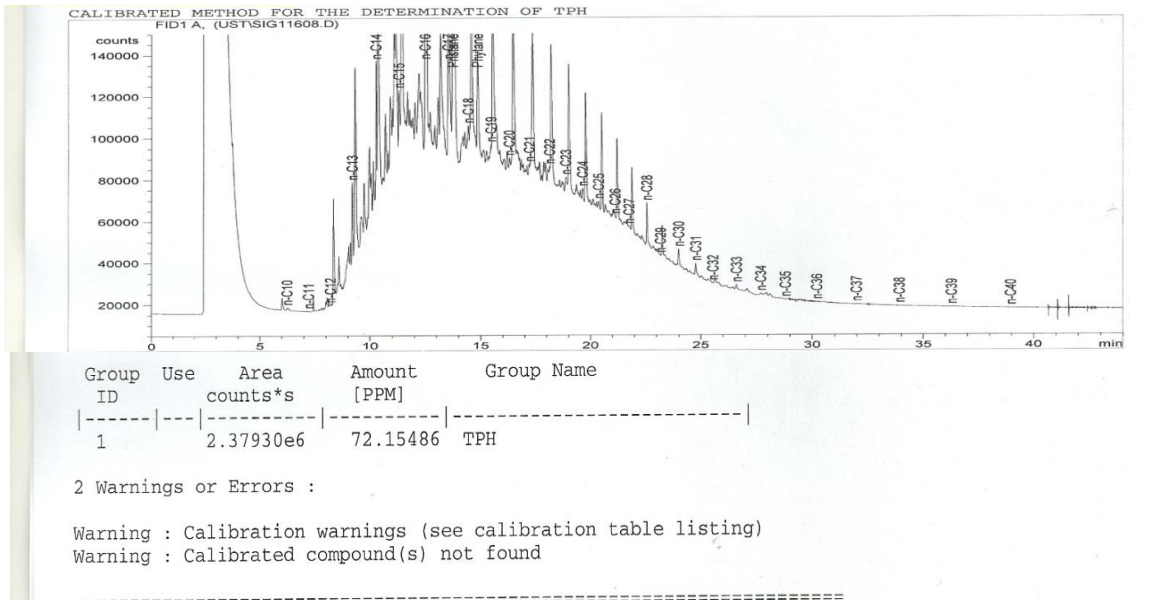
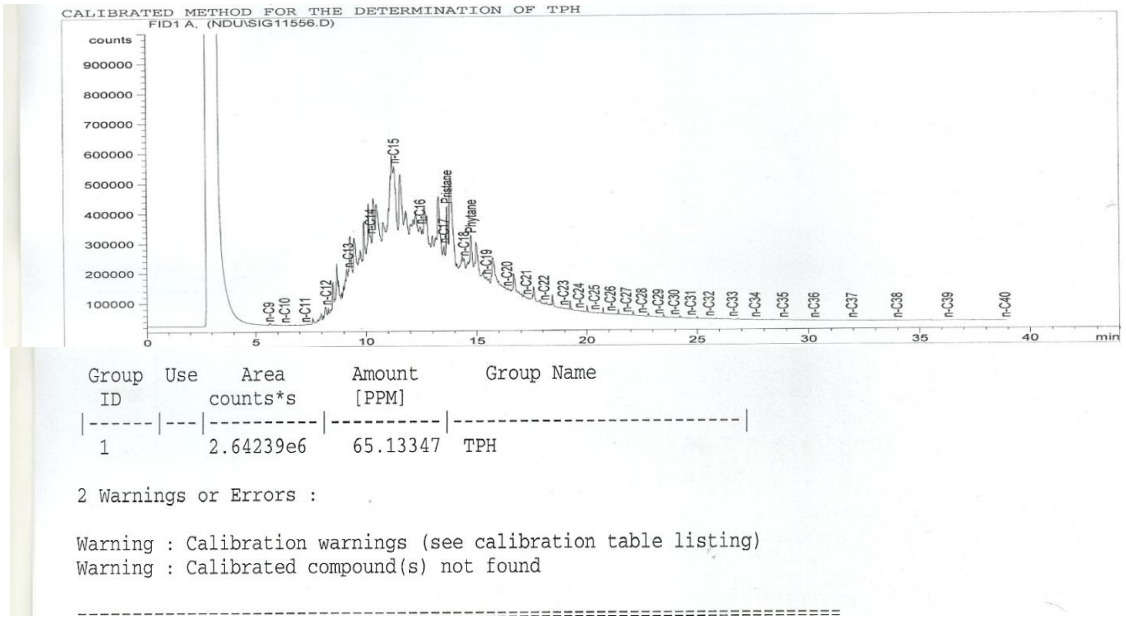


Fig. 6. Control of total petroleum hydrocarbon value and peaks on day 14 of crude oil polluted aquatic ecosystem using mineral salt broth

of 27.251 ppm to 0.046 ppm, 0.017 ppm and 0.035 ppm by Set-up 1, 2 and 3 respectively. Chain c21 was reduced from 1.211 ppm to 0.044 and 0.007 by Set-up 1 and 2 respectively. Chain c24 was slightly reduced from initial of 1.245 ppm to 0.997ppm, 0.368ppm and 0.356ppm by Set-up 1, 2 and 3 respectively. Chain c25 was degraded from initial of 0.938ppm to 0.847ppm, 0.256ppm and 0.042ppm by Set-up 1, 2 and 3 respectively. Chain c27 was also degraded from 2.557ppm to 0.292ppm, 0.046ppm and 0.089ppm by Set-up 1, 2 and 3 respectively. Chain c32 was reduced from initial of 0.279ppm to 0.016ppm, 0.007ppm and 0.011ppm by Set-up 1, 2 and 3 respectively.

Chain c35 was degraded from 1.744ppm to 0.021ppm, 0.002ppm and 0.023pm by Set-up 1, 2 and 3 respectively. Chain c37 was reduced from 5.851ppm to 0.020ppm and 0.008ppm by Set-up 1 and 3 respectively. Chain c40 was greatly degraded from 8.774ppm to 0.004ppm by Set-up 3 after Day 28. These reductions conform to other literatures [17,18,19] on the ability of organisms to effectively degrade petroleum hydrocarbons or organic pollutants.

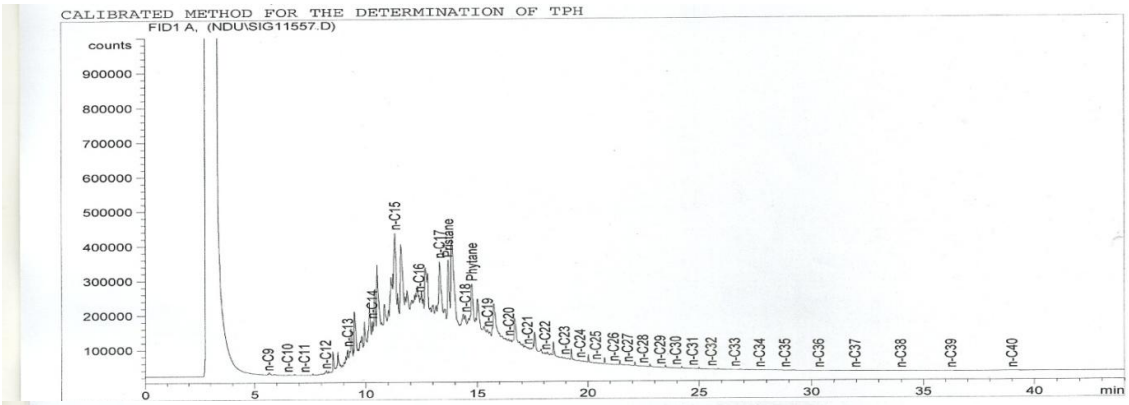


Group ID	Use	Area counts*s	Amount [PPM]	Group Name
1		2.64239e6	65.13347	TPH

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

Fig. 7. *Chryseobacterium sp.* of total petroleum hydrocarbon value and peaks on day 14 of crude oil polluted aquatic ecosystem using mineral salt broth

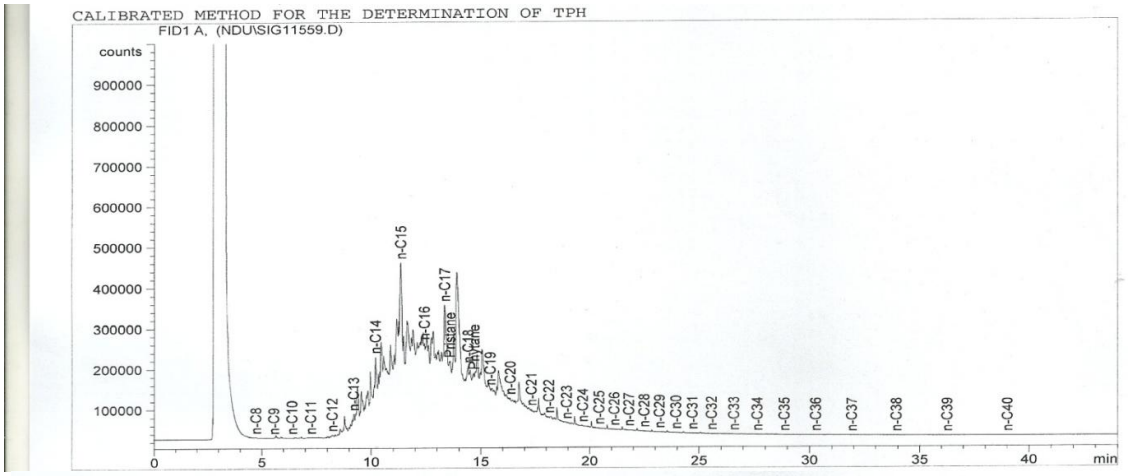


Group ID	Use	Area counts*s	Amount [PPM]	Group Name
1		4.38696e6	46.58154	TPH

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

Fig. 8. *Lysinibacillus fusiformis* of total petroleum hydrocarbon value and peaks on day 14 of crude oil polluted aquatic ecosystem using mineral salt broth

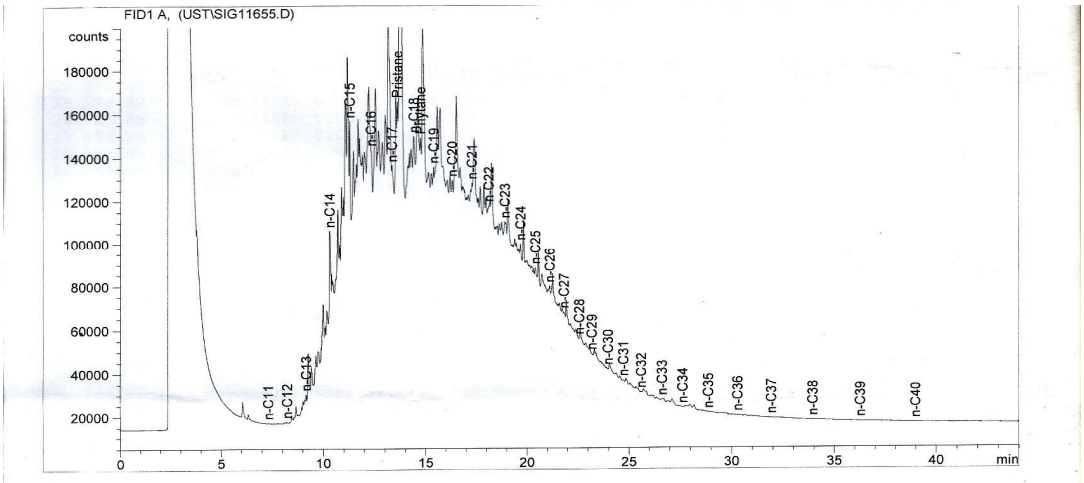


Group ID	Use	Area counts*s	Amount [PPM]	Group Name
1		2.80022e6	56.21648	TPH

1 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)

Fig. 9. *Chryseobacterium sp. and Lysinibacillus fusiformis* of Total Petroleum Hydrocarbon value and peaks on day 14 of crude oil polluted aquatic ecosystem using mineral salt broth



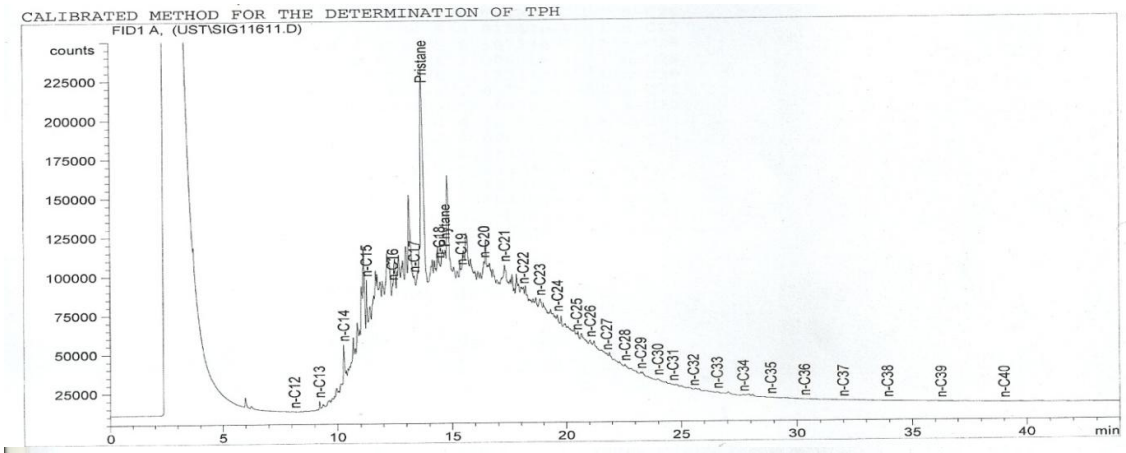
Totals : 71.21043

Results obtained with enhanced integrator!

Group summary :

Group ID	Use	Area counts*s	Amount [PPM]	Group Name
1		8.28641e5	71.21043	TPH

Fig. 10. Control of Total Petroleum Hydrocarbon value and peaks on day 28 of crude oil polluted aquatic ecosystem using mineral salt broth

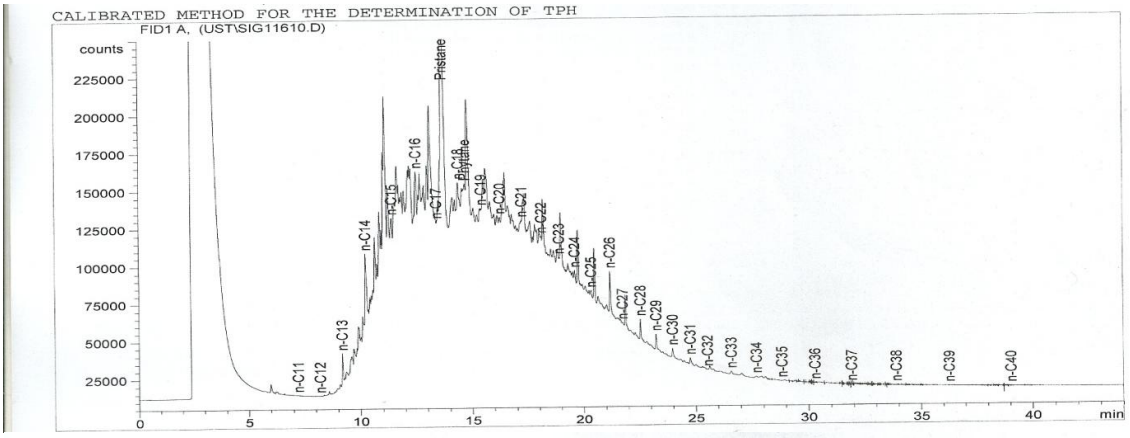


Group ID	Use	Area counts*s	Amount [PPM]	Group Name
1		1.62054e6	44.84597	TPH

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

Fig. 11. *Chryseobacterium sp.* of total petroleum hydrocarbon value and peaks on day 28 of crude oil polluted aquatic ecosystem using mineral salt broth



Group ID	Use	Area counts*s	Amount [PPM]	Group Name
1		2.82466e6	40.43799	TPH

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

Fig. 12. *Lysinibacillus fusiformis* of total petroleum hydrocarbon value and peaks on day 28 of crude oil polluted aquatic ecosystem using mineral salt broth

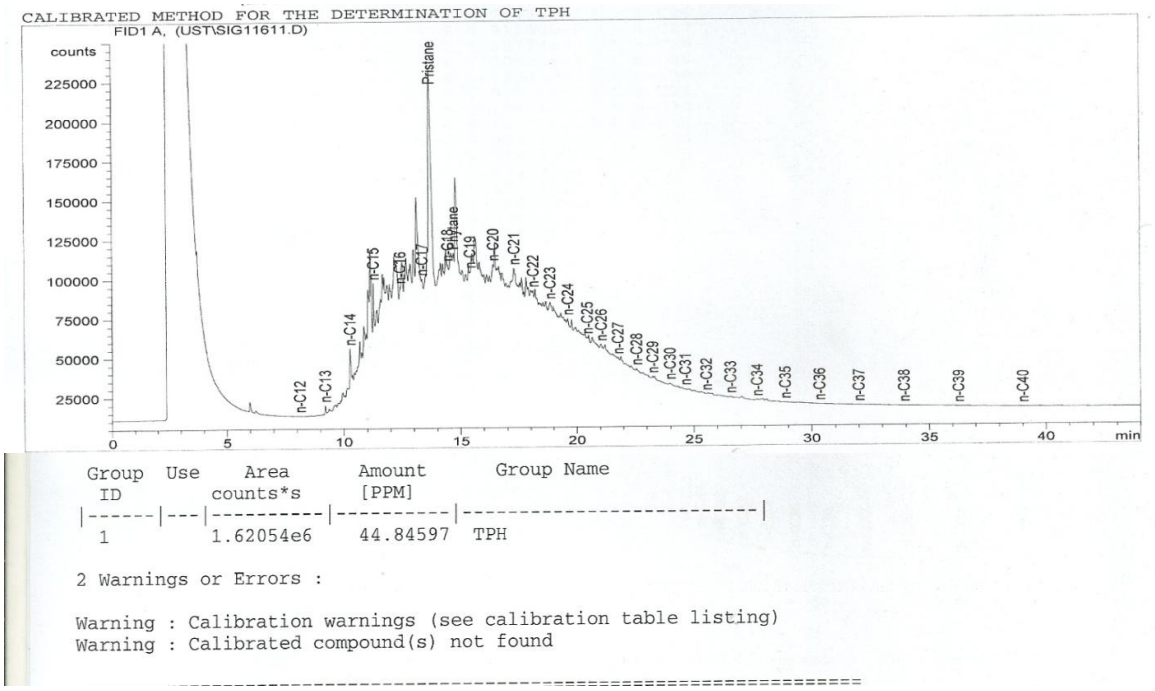


Fig. 13. *Chryseobacterium sp. and Lysinibacillus fusiformis* of total petroleum hydrocarbon value and peaks on day 28 of crude oil polluted aquatic ecosystem using mineral salt broth

4. CONCLUSION

From this research, it's can be concluded that microorganisms remain key players in a bid to remediate polluted lands and rid the environment of such toxic and mutagenic components. Biodegradation is known as a cost-effective and clean method compared to its physical and chemical counterparts. Although, *Lysinibacillus fusiformis* proved to be a better degrader as against *Chryseobacterium sp.* and the mixed culture; the study showed that a range of bacterial genera is capable of effectively degrading hydrocarbon components with the release of suitable and reusable end products.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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