

Full Length Research Paper

Biodegradation of hydrocarbon by *Enterobacter* sp IAA-01 isolated from hydrocarbon exploration site soil of Kukawa Northeastern Nigeria

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The accidental release of diesel and other hydrocarbon to the environment lead to the pollution of the environment. The search for an environmentally friendly technique for the remediation of such polluted environment is not to be overemphasis. Microorganisms are used in the bioremediation of polluted environments. Hydrocarbon-degrading bacterium isolated from the hydrocarbon exploration site soil of Kukawa North-eastern Nigeria, and was studied for its biodegradation potential of diesel. Culture base techniques using nutrient agar and mineral salt medium supplemented with 1% diesel was used. The bacterium isolate was identified as *Enterobacter* sp. strain IAA-01. Identification was carried out using 16S rRNA sequencing and molecular phylogeny analysis using the Phylip software. Gravimetric analysis was conducted to measure the percentage biodegradation of the bacterium for 21 days, and confirmed using Gas chromatography (GC) analysis where the bacterium degraded diesel hydrocarbon 78% after 21 days and total degradation was revealed in GC analysis after 21 days. The results prevailed the ability of *Enterobacter* sp. strain IAA-01 effectively biodegrade diesel oil.

Key words: *Enterobacter* sp., biodegradation, gas chromatography, pollution.

INTRODUCTION

The intense increase in production, refining and distribution of crude oil and its products has conveyed with it an ever accumulative problem of environmental pollution (Afuwale and Modi, 2012).

The problem of environmental pollution due to oil and oil products is not limited only to oil producing countries

but countries with large number of refineries and refining capacity such as Nigeria where the crude oil are being transported and refined, especially through oil producing region are susceptible to oil pollution (Oboh et al., 2006). The regions are characterised by detached, indiscriminate and highly unregulated disposal of petroleum products.

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Illustrious that even small release of petroleum hydrocarbons into aquifers can lead to concentrations of dissolved hydrocarbons far in excess of regulatory limits (Onifade and Abubakar, 2007). Apart from the indiscriminate disposals of oil there are also hydrocarbon pollution due to oil spills especially diesels and gasoline in Nigeria. Research findings have shown statistically significant impact of such reckless disposal on the ecosystem (Bayode et al., 2011). However, various remediation methods were employed to remediate hydrocarbon polluted soils and water, and the biological method turn to be environment friendly and cost effective, studies have shown that hydrocarbon degrading bacteria are naturally present in these environments and play a very important role in the removal of the pollutants (Allamin et al., 2014). Several species of the genus *Enterobacter*, *Bacillus*, *Micrococcus*, *Nocardia*, *Corynebacterium*, *Pseudomonas*, *Flavobacterium*, *Achromobacter*, *Alcaligenes* and *Proteus* are some of the commonly isolated degraders (Chikere et al., 2009; Afuwale and Modi, 2012). The main objective of this work is to isolate and screen bacteria with the ability to degrade hydrocarbon maximally and subject it to molecular characterization.

MATERIALS AND METHODS

Study area

The study area is Kukawa, one of the 27 local governments in Borno State Nigeria. Kukawa is located in the northern part of Borno bordering four other local governments of Abadam, Monguno, Guzamala and Marte. It also has international border with Chad with geographical coordinates 12°55'33" North and 13°34'12" East (Figure 1). It has an average elevation/altitude of 277 meters. The majority populations are into farming and fishing with estimated population of over 25,000. Kukawa is considered one of the potential crude oil prospecting area by the Nigerian National Petroleum Corporation (NNPC) (WFP 2018).

Sample site

Soil samples were collected from the exploration sites of Kukawa where Hydrocarbon exploration is taking place (Rasheed et al., 2012). The soil samples were collected with sterile trowel after clearing debris from the soil surface. Samples were collected in sampling bottles and were transported to the laboratory for further analysis (Waheed et al., 2018).

Microbiological analysis of soil sample

One gram each of the samples was weighed aseptically and placed into test tube containing 9 ml of distilled water the test tube was shaken vigorously in order to dislodge the microorganisms that adhered to the soil particles (Brito et al., 2006). The content of the tube was serially diluted. Aliquot (0.1 ml) from dilution (10^{-5}) was spread in triplicates on MSM modified and supplemented with 1%

diesel ($(\text{NH}_4)_2\text{SO}_4$ 0.1 g, K_2HPO_4 0.1 g, CaSO_4 0.05 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.2 g, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.01 g, and distilled water 1.0 L, pH 7.0 with 1% diesel) oil agar (OA) for the enumeration of oil utilizing bacteria.

The plates were incubated at room temperature ($30 \pm 2^\circ\text{C}$) for 5 to 7 days. The colonies developed on the plates were counted and recorded as colony forming units per gram (cfu/g) of soil. Pure cultures of the isolates were obtained by repeated sub-culturing on media used for primary isolation. The pure isolates were obtained on agar slant for further characterization (Holts and Williams, 1994).

The rate of diesel oil degradation by selected microbial isolates was determined using gravimetric analysis and gas chromatographic spectrophotometric (GC) analysis techniques. One milliliter of nutrient broth grown isolates was inoculated into 100 ml of mineral salts medium in Erlenmeyer flask. Then 0.5 ml of diesel oil was supplemented as carbon source to each Erlenmeyer flask. Control flask contained no added organism, and then it was incubated on a shaking incubator at 150 rpm for 21 days. At 7 days interval, flasks per organism were removed and the amount of oil left was determined by extracting the residual oil with n-hexane gravimetrically using a weighing balance and the optical density was measured by noting the absorbance reading at 600nm wavelength in a spectrophotometer. The amount of diesel oil degraded was calculated using the formula (Latha and Kalaivani 2012):

$$\% \text{ biodegradation} = \frac{\text{Control} - \text{Degraded}}{\text{Degraded}} \times 100 \quad (1)$$

Molecular identification of the strain

The genomic DNA was extracted using a commercial kit (GeneJet Genomic DNA purification kit, ThermoScientific, Lithuania) and amplified using the following PCR universal primers; reverse: 5'-TAC GGT TAC CTT GTT ACG ACT T-3' and forward: 5'-AGA GTT TGA TCC TGG CTC AG-3'. PCR was carried out under the following conditions: 1st cycle at 96°C for 4 min as an initial denaturation; 30 cycles at 94°C for 1 min for denaturing followed by an annealing stage at 58°C for 1 min, an extension stage carried out at 72°C for 1 min and a final extension at 72°C for 7 min. The sequence was further deposited at the NCBI Gen bank and assigned an accession number of MG651780. For the phylogenetic tree analysis, twenty 16s rRNA sequences were obtained from Genbank showing the closest identity to *Enterobacter* species. The evolutionary analysis was carried out using MEGA6. A Neighbour-Joining method involving closest nucleotide sequences sourced from the BLASTn exercise was utilised to infer evolutionary history. The Maximum Composite Likelihood method was utilised to calculate the evolutionary distances. In the analysis, codon positions included were the 1st+2nd+3rd. Also, missing data and gaps were removed from all positions resulting in a final 1353 positions presented in the final dataset.

RESULTS

Molecular identification of the bacterium and characterization

Isolate IAA-01 was identified using a molecular phylogenetic analysis of the 16S rDNA sequence. Molecular identification begins with a BLASTn exercise on the NCBI Gen Bank database. The result shows a

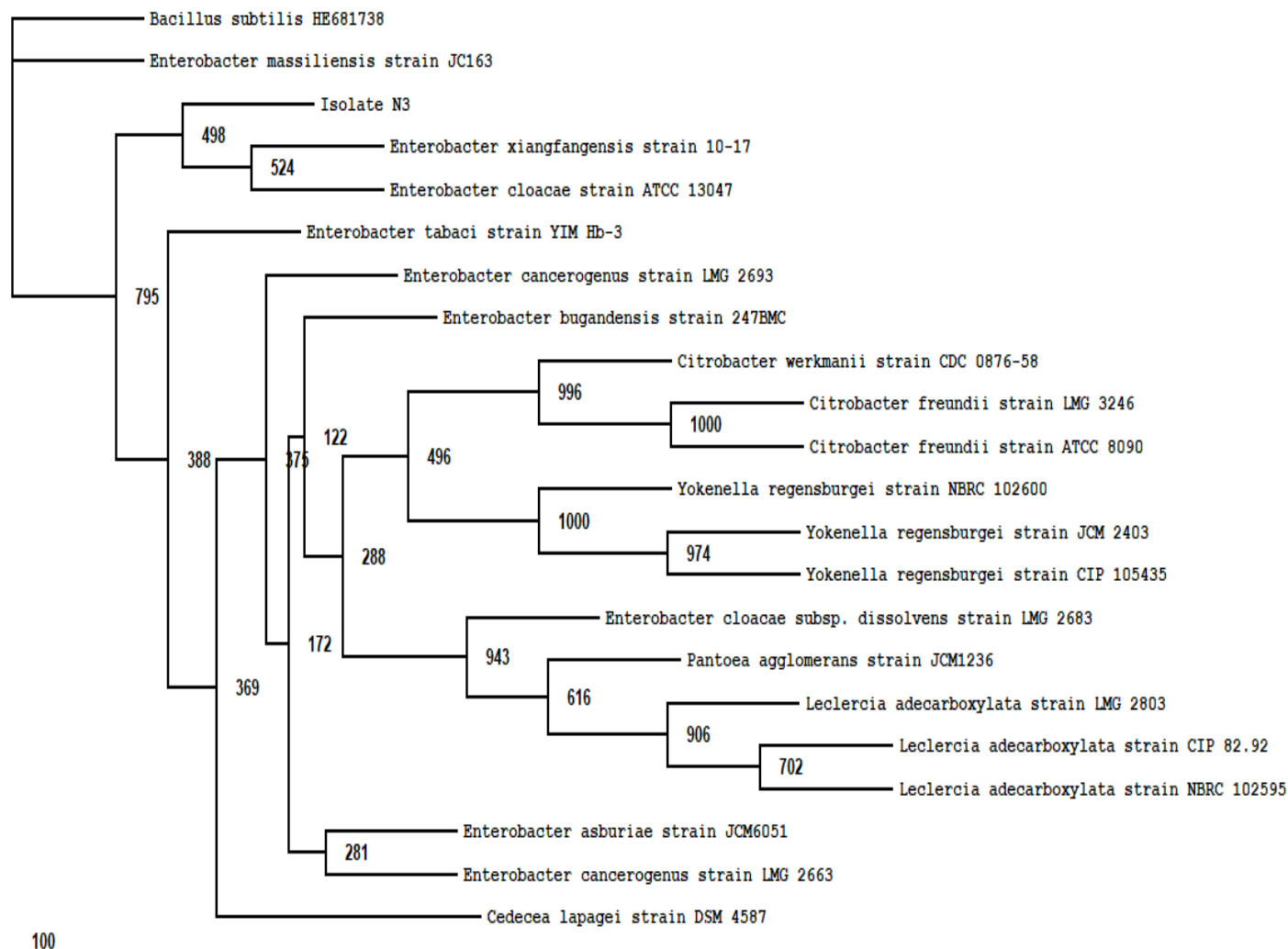


Figure 1. Neighbour-joining tree based on partial 16s rRNA sequence demonstrating the phylogenetic relatedness of strain IAA-01 to other bacterial species. *Bacillus subtilis* strain GO19 HE681738 as outgroup.

96% similarity to *Enterobacter* spp. The percentage of replicate trees (1000 replicates) is shown next to the branches (Figure 1) based on a bootstrap exercise. The bacterium is linked to several *Enterobacter* species clades such as *Enterobacter cloacae* but with low bootstrap values. At this stage, the bacterium was tentatively identified as *Enterobacter* sp. IAA-01.

The result of the study shows the biodegradation activity of hydrocarbon by *Enterobacter* sp. IAA-01 over time, from 0 days to 21 days. The bacterium was able to degrade 78.0% of diesel hydrocarbon after 21 days (Figure 2). The bacterial growth was also consistent to the biodegradation with optical density (O.D) of 0.94 at 600nm wavelength at 21 days (Figure 2). The GC analysis shows the extent of degradation of the

hydrocarbons over time of 0 day to 14 days with chromatographic peaks indicating degree of degradation by *Enterobacter* sp. IAA-01 where the first peak was for the solvent used for the analysis, whereas the subsequent peaks represent the hydrocarbon in sample (Figures 3 to 5).

DISCUSSION

Bacterial species isolated was screened in order to find out their biodegradation potentials. *Enterobacter* sp. IAA-01 was able to utilize hydrocarbons as the sole source of carbon luxuriantly.

The bacteria had been reported by Ajayi et al. (2008)

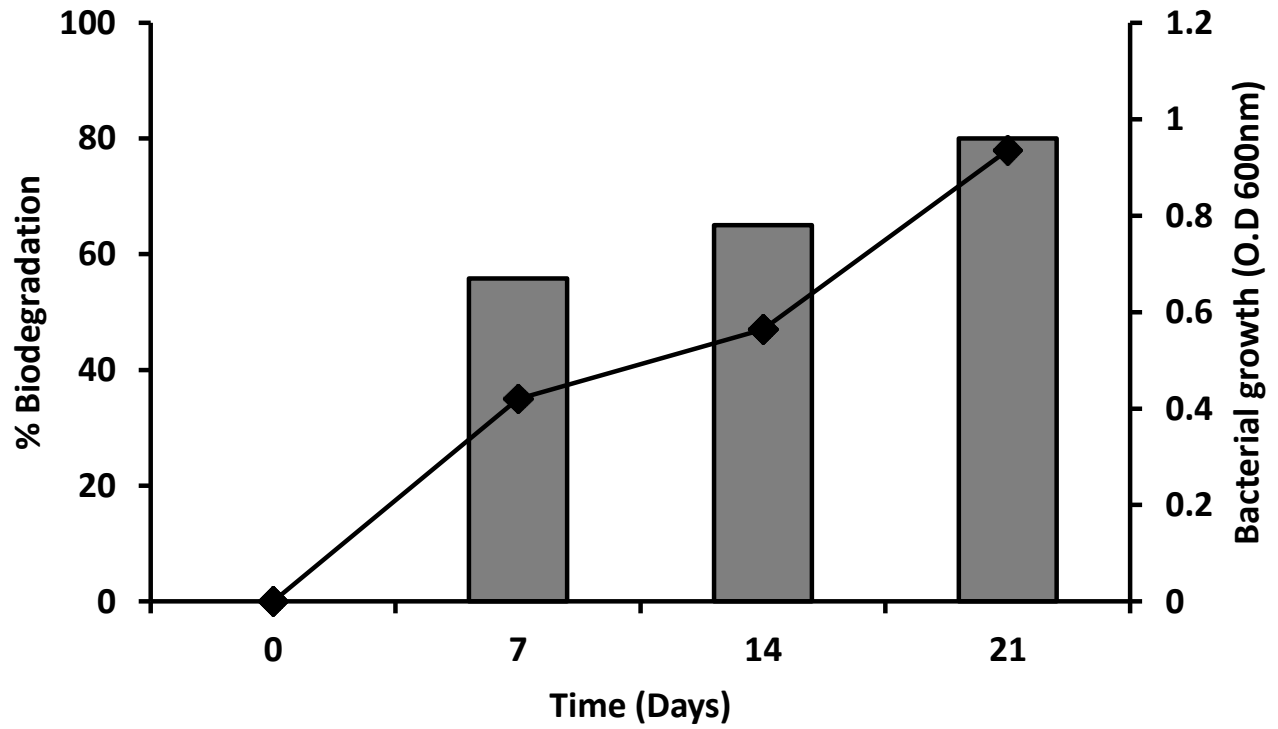


Figure 2. The percentage biodegradation and growth of *Enterobacter* sp. IAA-01 over time (0-21 days).

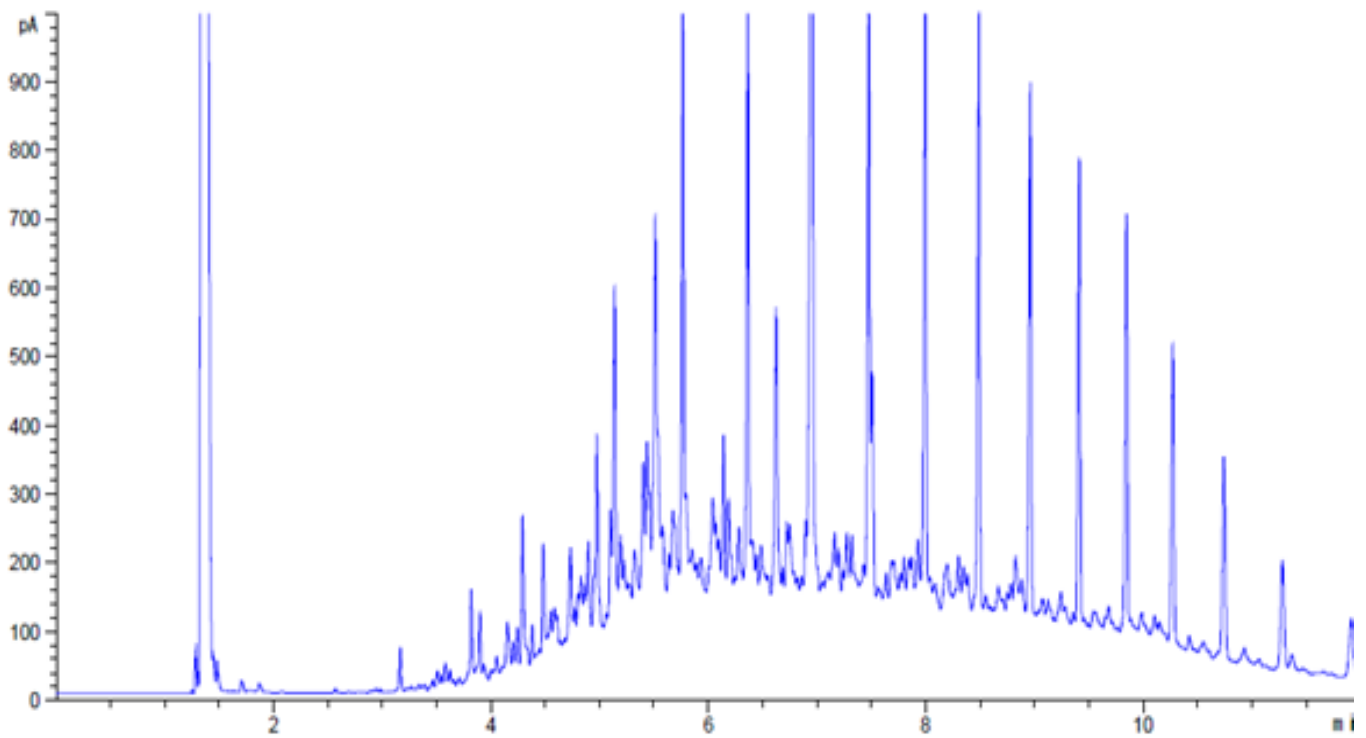


Figure 3. Chromatography peaks of hydrocarbon showing degradation of hydrocarbon constituent by *Enterobacter* sp IAA-01 in 0 days; X axis intensity count in (pA) Y- axis time (minutes).

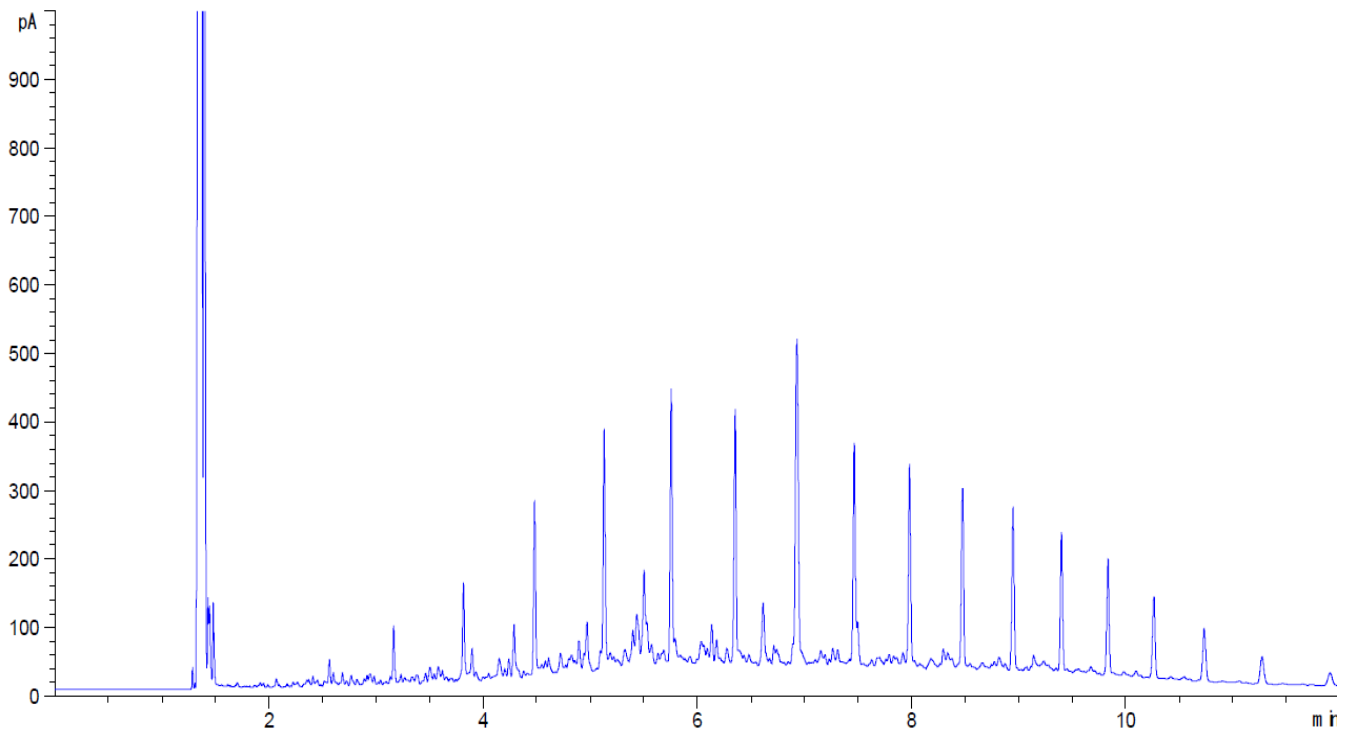


Figure 4. Chromatography peaks of hydrocarbon showing degradation of hydrocarbon constituent by *Enterobacter* sp IAA-01 in 7 days; X axis intensity count in (pA) Y- axis time (minutes).

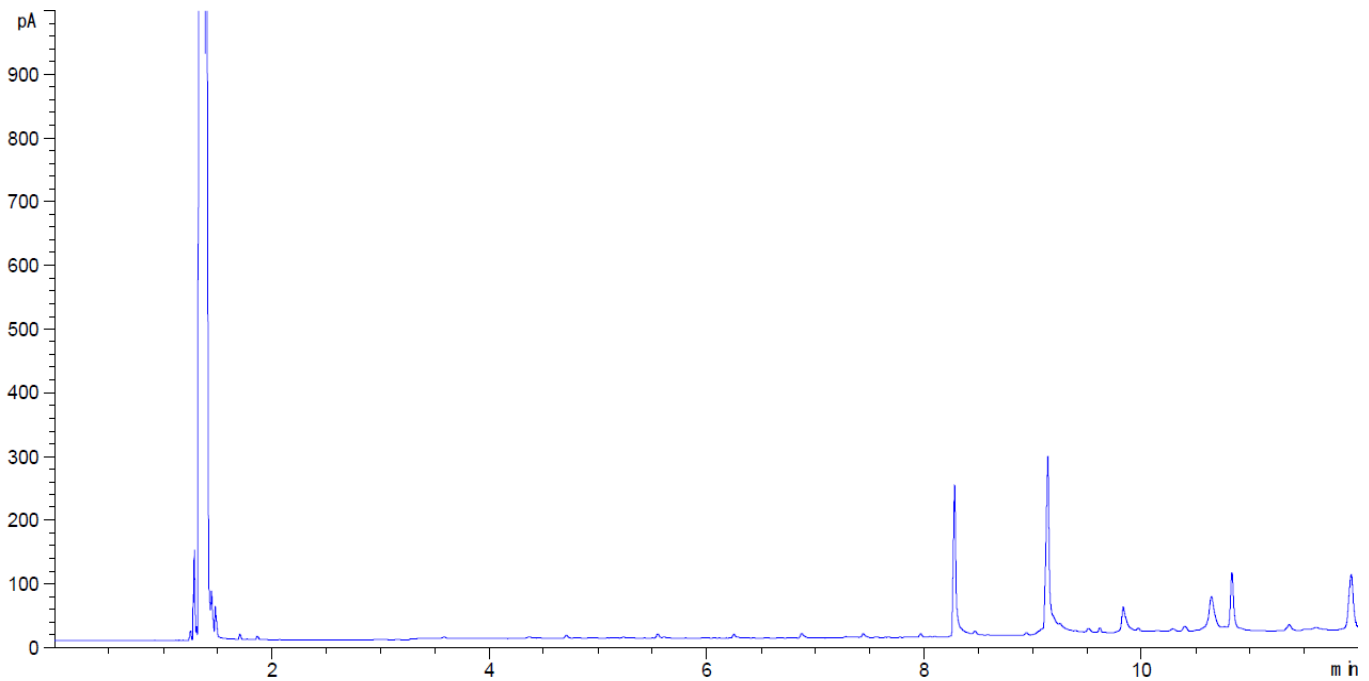


Figure 5. Chromatography peaks of hydrocarbon showing degradation of hydrocarbon constituent by *Enterobacter* sp IAA-01 in 14 days; X axis intensity count in (pA) Y- axis time (minutes).

as well as Malik and Ahmed (2012), as efficient hydrocarbon degraders. This means that *Enterobacter* sp IAA-01 used the hydrocarbon as a source of carbon and energy. The variation in the capacity of the isolates to utilize crude oil could be due to differences in the competence of hydrocarbon degrading enzyme. Generally, soils receiving micro-seepage of hydrocarbon tend to be dominated by bacteria utilizing hydrocarbon in a large number as reported by (Hitzman 1959; Klusman and Saeed, 1996; Leifer and Judd, 2002; Bashir, 2012). And their ability to withstand the toxic effect of the hydrocarbon constituents.

The study revealed that *Enterobacter* sp IAA-01 caused 78.0% oil degradation after 21 days, (Figure 2). The efficient ability of the bacteria in degrading hydrocarbon has been reported by other investigators (Chikere et al., 2009; Afuwale and Modi, 2012). The results of GC analysis further confirmed the biodegradation potential of *Enterobacter* sp IAA-01 and revealed that the bacteria is capable of utilizing most of the hydrocarbon components especially straight chain alkanes, and cyclo-alkanes (Figures 3 to 5). Generally, the accepted pattern of susceptibility of hydrocarbon components to microbial degradation is n-alkane > branched alkanes > low-molecular weight aromatics > polycyclics (Bogan et al., 2003). However, system-specific exceptions to this pattern have been found (Makut and Ishaya, 2010). The GC analysis of the 0 day (Figure 3) comparatively shows non-degradation of all hydrocarbon components. Figure 4 shows hydrocarbon degradation pattern by, in which most of the alkanes and branch alkanes were utilized by the bacteria (Allamin et al., 2020), the remaining were aromatic compounds, which were difficult to degrade.

Conclusion

It can be concluded from these results that *Enterobacter* sp IAA-01 used in this study had considerably high ability of degrading hydrocarbon, because over time most of the chromatographic peaks of the hydrocarbon were utilized by the bacteria.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest

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REFERENCES

- Afuwale C, Modi HA (2012). Study of bacterial diversity of crude oil degrading bacteria isolated from crude oil contaminated sites. *Life sciences Leaflets* 6:13-23.
- Ajayi AO, Balogun SA, Adegbehingbe K (2008). Microorganisms in the crude oil-producing areas of Ondo State, *Nigeria*. *Academic Journals Scientific Research and Essay* 3(5):174-179.
- Allamin IA, Halmi MIE, Yasid NA, Ahmad SA, Abdullah SRS, Shukor Y (2020). Rhizodegradation of petroleum oily sludge-contaminated soil using *Cajanus cajan* increases the diversity of soil microbial community. *Scientific Reports* 10(1):1-11.
- Allamin IA, Ijah UJJ, Ismail HY, Riskuwa ML (2014). Occurrence Of Hydrocarbon Degrading Bacteria In Soil In Kukawa, Borno State. *International Journal of Environment* 3(2):2091-2854.
- Bashir AH (2012). Diversity and morphology of bacterial community characterized in topsoil samples from the Gaza Strip, Palestine. *Research Journal of Microbiology* 7(6):309-318.
- Bayode OJ, Adewunmi EA, Odunwale S (2011). Environmental implications of oil exploration and exploitation in the coastal region of Ondo State, Nigeria: A regional planning appraisal. *Journal of Geography and Regional Planning* 4(3):110-121.
- Bogan BW, Lahner LM, Sullivan Beilen WR, Paterek JR (2003). Degradation of straight chain aliphatic and high – molecular weight polycyclic aromatic hydrocarbons by a strain of *Mycobacterium austroafricanum*. *Journal of Applied Microbiology* 94:230-239.
- Brito EM, Ranchou-Peyruse A, Verbaere A (2006). Characterization of hydrocarbonoclastic bacterial communities from mangrove sediments in Guanabara Bay, Brazil. *Research in Microbiology* 4(6):344-352.
- Chikere CB, Okpokwasili GC, Chikere BO (2009). Bacterial diversity in a tropical crude oil-polluted soil undergoing bioremediation. *African Journal of Biotechnology* 8(11):2535-2540.
- Hitzman DO (1959). Prospecting for petroleum deposits. US Patent 2880142.
- Holt JG, Williams W (1994) *Bergey's Manual of Determinative Bacteriology* (1994). 9th edition, Baltimore. *Advances in Applied Science Research* 3(5):2789-2795.
- Klusman R, Saeed M (1996). Comparison of light hydrocarbon microseepage mechanisms, in: *Hydrocarbon Migration and Its near Surface Expression*, edited by: Schumacher, D. and Abrams, M., AAPG Memoir, Tulsa pp.157-168.
- Latha R, Kalaivani R (2012). Bacterial Degradation of Crude Oil by Gravimetric Analysis. *Journal of Microbiology and Biotechnology* 6:34-39.
- Leifer I, Judd AG (2002). Oceanic methane layers: the hydrocarbon seep bubble deposition hypothesis. *Terra Nova* 14:417-424.
- Malik ZA, Ahmed S (2012) Degradation of petroleum hydrocarbons by oil field isolated bacterial consortium *African Journal of Biotechnology* 11(3):650-658.
- Oboh BO, Ilori O, Akinyemi O, Adebuseye SO (2006). Hydrocarbon Degrading Potentials of Bacteria Isolated from a Nigerian Bitumen (Tarsand) Deposit. *Nature and Science* 4:3.
- Onifade OA, Abubakar FA (2007). Characterization of Hydrocarbon-Degrading microorganisms From Isolated from Crude Oil Contaminated Soil and Remediation of the Soil by Enhanced Natural Attenuation. *Research Journal of Microbiology* 2(2):149-155.
- Rasheed MA, Lakshmi M, Kalpana MS, Patil DJ, Dayal AM, Raju SV (2012). Evaluation of hydrocarbon charge using microbial and light gaseous hydrocarbon anomalies in the Seabed Sediment Samples of Gulf of Mannar, Kerala-Konkan Offshore Basin *Natural Resources Research* DOI: 10.1007/s11053-012-9189-x.
- Waheed SM, Sangal P, Shukla S (2018). Microbiological analysis for hydrocarbon exploration. *Environmental Risk Assessment and Remediation* 2(3):1-7.
- World Food Programme (WFP) (2018). Rapid food security Assessment in Doro Baga, Baga, Kukawa and Cross Kauwa (Kukawa LGA).