# GC-MS Profiles of Spent Engine Oil Biodegradation: Influence of *Bacillus* Species Isolated from Oil Contaminated Soil of Auto Mechanic Workshop

## Ikuesan F. A.1\* Okonji H. I.1 and Adebayo A. J.2

- 1. Department of Biological Sciences, Olusegun Agagu University of Science and Technology, Okitipupa, Nigeria.
- 2. Department of Chemical Sciences, Olusegun Agagu University of Science and Technology, Okitipupa, Nigeria
  - \* Corresponding author: fa.ikuesan@oaustech.edu.ng

Abstract: Spent engine oil, a petroleum hydrocarbon, is often indiscriminately released into the environment thereby posing significant risks to both ecosystems and human health. This study evaluated the biodegradation potential of Bacillus species isolated from oil-contaminated soil. Soil samples were aseptically collected from an auto-mechanic workshop in Okitipupa, Nigeria, and analyzed using standard microbiological procedures and Gas Chromatography-Mass Spectrophotometry (GC-MS) method. The load of total heterotrophic and hydrocarbon-degrading bacteria in the assayed sample were  $4.5 \times 10^4$  and  $3.9 \times 10^3$  CFU/g, respectively. The isolates were identified as *Bacillus niacini* and *Bacillus circulans*, with similarities of 90.4% and 87.9%, respectively, confirmed using Advanced Bacterial Identification Software. Biodegradation experiments were conducted using 24-hour-old broth cultures of the isolates in Bushnell-Hass medium supplemented with 1% (v/v) spent engine oil. After a fourteen-day incubation period, GC-MS profile was performed to assess biodegradation. GC-MS profiling of untreated samples revealed high molecular weight hydrocarbons, including n-Docosanoic acid methyl ester (C23H46O2) and 11,14-Eicosadienoic acid methyl ester (C21H38O2). In contrast, treated samples exhibited significant degradation, with the presence of lower molecular weight compounds such as 2,5,5-Trimethyl-1,6heptadiene (C<sub>10</sub>H<sub>18</sub>) and Hendecane (C<sub>11</sub>H<sub>24</sub>). These changes in molecular weight, retention time, peak area, and chemical structure confirm the effective metabolic activity of both bacterial strains. The results demonstrate that Bacillus niacini and Bacillus circulans possess robust biodegradation capabilities, enabling them to transform complex and potentially hazardous hydrocarbons into simpler, less toxic compounds. These species hold strong potential for use in bioremediation of environments contaminated with spent engine oil and related pollutants. Further research is recommended to optimize degradation conditions and evaluate large-scale field applications to support practical environmental cleanup initiatives. Key word: Biodegradation, Oil degrading bacteria, Oil polluted soil, Petroleum hydrocarbon, Spent engine oil

## **INTRODUCTION**

etroleum and its derivatives important energy sources that drive our normal activities for energy and economy. essential needs The and uncontrolled release of petroleum derived products results in environmental pollution of varying intensity with worrisome impact of universal concern on every component of the environment and ecosystem health in (Ikuesan *et al.*, 2022). persistence of petroleum pollutants in the environment is dependent on the quantity and quality of the spilled xenobiotics and the characteristics of the ecosystem receiving the hydrocarbon (Adeleye et al., 2018; Ikuesan, 2025).

Spent engine oil is liquid, black brown in appearance that is collected from various internal combustion engines including all

categories of automobiles, industrial machines and generator after servicing. Bahadure et al. (2013) reported that spent engine oil (SEO) contains considerable amounts of long chain saturated hydrocarbons (80 - 90%) and a liquid blended of varying molecular weight (C15 – C18), aliphatic and aromatic hydrocarbons other constituents chlorodibenzofurans, polychlorinated biphenyl and additives. Spent engine oil also referred to as used engine oil is among the several petroleum hydrocarbons (PHs) frequently and indiscriminately discharged into the environment and therefore, constitute significant source of pollution environmental without consideration for the consequences. Soil pollution with hydrocarbons emanating from human activities have caused devastating impacts on the soil ecosystems as well as soil microbial functions. due to their complex relative nature. stability, persistence and non-degradability (Agarry and Ogunleye, 2012). Ikuesan (2017) reported that the consequence of crude oil on soil microorganisms is influenced by the amount of oil spilled and contact time. Bioremediation and variety a physicochemical treatments are among the several initiatives that have been devised to remove petroleum hydrocarbon contaminants released into the environment as conscious steps to ameliorate the risks imposed by PH contamination on the ecosystem and human health (Das and Chadran, 2011). Although, the application of these physicochemical methods is effective in pollutant removal, but these methods often result in the generation of other toxic environmental pollutants in addition to the high cost of resources involved in their applications. Bioremediation has therefore received enormous acceptability among environmental scientists because it is cost effective with simplicity of procedure and applicability over a vast area of polluted site. In this biological process. microorganisms with catabolic potential utilize PHs as source of carbon and energy thereby transforming them into innocuous substances including microbial biomass and carbon dioxide which are all safe and environmentally friendly (Ekpenyong and Antai, 2007). The nature and amount of hydrocarbon spilled, temperature, nutrient, moisture, aeration and pH and population of autochthonous type microbial and introduced community or exogenous microorganisms are among the parameters influencing the rate of biodegradation (Das and Chandran, 2011; Odeyemi, 2014). microorganisms Different have been implicated in the degradation of complex hydrocarbons in soil using diverse mechanisms, although, their biodegradation potential varies. The capability of diverse bacterial genera to degrade petroleum in soil has been extensively products reported by several authors (Uba and

2013) degradation Ifeanyi, and of hydrocarbons cannot succeed without these hydrocarbonoclastic ofmicroorganisms (Adeleye et al., 2018). The introduction of identified oil degraders with high catabolic capabilities to the polluted environment will promote synergistic cooperation interactions and metabolic among different microbial species and enhance the degradation capabilities of the microbial community (Ikuesan et al., 2015; Boboye et al., 2023; Love et al., 2023). Bacillus species have been attracting interest in environmental bioremediation strategies due to their several characteristics that make them more beneficial in the biodegradation of petroleum hydrocarbons (Nurulhuda et al., 2018). The influence of Bacillus species biodegradation of petroleum on hydrocarbons can be attributed to several such as the production factors biosurfactant which are potent emulsifiers for enhancing bioavailability of petroleum hydrocarbons to microbial attack. Bacillus species exhibit tolerance and adaptability to extreme environments, robust metabolic versatility, biofilm formation on pollutant surfaces. Other factors include Bacillus species exhibit a diverse array of enzymes (alkane hydroxylases, monooxygenases dioxygenases) which enhances capabilities to attack and breakdown complex pollutants including petroleum hydrocarbons into less harmful compounds, metabolic pathways, spore formation and genetic adaptations (Sahal., 2023, Das et al., 2024), thus, making Bacillus species the major source of potential candidates for hydrocarbon degradation. These bacteria are known for their versatility and metabolic capabilities, allowing them to thrive in environments, diverse including contaminated with hydrocarbons. Nevertheless, information on the use of Bacillus species in the degradation of soil polluted with spent engine oil is less studied, thus necessitating this study. Therefore, this study aims to investigate the use of Bacillus species in the degradation of complex

hydrocarbons in the spent engine oil polluted soil.

## MATERIALS AND METHODS

Collection of Soil Sample: Spent engine oil-contaminated soil sample was aseptically collected into sterile polythene bag from three points at about 10.00 am from a mechanic workshop in Okitipupa, Nigeria using sterile hand auger and then transported in ice chest maintained at 4° C to the laboratory for analysis within 6 hr of collection.

Preparation of culture media: Culture media and test solutions were prepared according to manufacturer's specification. Twenty-eight grams (28 g) of nutrient agar was dissolved in 1 liter of distilled water in a conical flask while Bushnell-Hass mineral salt medium was composed with 0.20 g magnesium sulphate, 0.02 g calcium chloride, 1.00 g monopotassium phosphate, 1.00 g dipotassium phosphate, 1.00 g ammonium nitrate, 0.05 g ferric chloride and 20.00 g agar and then dispensed into 1 liter of sterile distilled water and pH was adjusted to 7.2. The conical flask containing the media were then plugged with cotton wool and wrapped with aluminum foil after thorough mixing with the aid of a magnetic stirrer. Exactly 20 ml of each prepared medium was dispensed into MacCartney bottles, autoclaved at 121°C for 15 minutes and maintained at about 44°C. The MSM medium was also fortified with fungisol (10 mg/l) after sterilization and spent engine oil (1% w/w) sterilized using 0.45 µm Millipore filter was added to serve as carbon and energy source.

Enumeration of bacterial population in the assayed soil sample: The pour plate technique was employed for the enumeration of bacteria from the spent engine oil-contaminated soil samples. One gram (1 g) of homogenized composite soil was aseptically dispensed into 9 ml of sterile distilled water, mixed thoroughly to ensure dislodgement and even distribution of microorganisms in the sterile distilled water to form a stock solution and then serially

diluted to the 7th dilution. Exactly one milliliter (1 ml) aliquot from dilutions 10<sup>-3</sup> to 10<sup>-7</sup> was inoculated into sterile Petri dishes and then separately overlaid with nutrient agar and Bushnell-Haas media to determine heterotrophic (THB) the total hydrocarbon-utilizing bacteria (HUB) and incubated at 35°C for 48 hours and 14 days for nutrient agar and Bushnell-Hass media for the enumeration of THB and HUB respectively (Ikuesan et al., 2015). The plates were observed for growth and selected for count after the expiration of the incubation period. The culture plates in which the number of colonies were 30-300 and its triplicates was selected and counted. The averaged count was then expressed as colony forming unit per gram (CFU/g) of sample.

Characterization and identification of Bacillus species: Colonies of hydrocarbon utilizing bacteria on BHM were purified by repeated streaking on freshly prepared nutrient agar and then identified using cultural, morphological and biochemical characteristics, including catalase test, citrate test, hydrogen sulphide (H<sub>2</sub>S) production, indole, urease, methyl red, Voges-Proskauer test, motility, starch hydrolysis and sugar fermentation following the procedures described by Cheesbrough (2006). The characteristics of suspected Bacillus species compared then with standards obtainable using the Advanced Bacterial Identification Software (Sorescu and Stoica, 2021).

GC-MS-assisted profiling of spent engine-culture medium of bacterial isolates: The identified Bacillus species were grown in nutrient broth medium for 24 hours and seeded into a flask containing 100 ml of Bushnell-Haas broth medium, followed by the addition of 1% (v/v) pre- sterilized spent engine oil as carbon and energy source. Then, the flasks were incubated at 35°C for 14 days in a rotary shaker at 150 rpm. After the incubation period, the content of the flasks was centrifuged at 2,000 rpm for 1 hour and filtered to obtain supernatant. The control sample and cell-free suspension used

engine oil residue, were taken for GCMS (QP2010 Plus Shimadzu, Japan) analysis using the capillary column (Alnuaimi *et al.*, 2020) following the modified method of Elkemary *et al.* (2023). This involved the use of Helium as carrier gas at a flow rate of 20.8 ml/minute and a split ratio of 10.0 by using the temperature program: 55°C for 2 minutes; rising at 120.0°C/minutes to 220°C and held for 3 minutes. The injector was held at 250°C. The mass spectrometer was obtained using electron ionization (EI) at 70 electrons volts (eV) from 18 to 600 m/z.

## Statistical analysis of data obtained

The analysis of the data from this study was performed using the Statistical Package for the Social Sciences (SPSS; version 6.0). A one-way analysis of variance (ANOVA) was performed followed by Duncan's test at a 5% level of significance to determine the differences between the means.

#### **RESULTS**

Table 1 revealed varying population of total heterotrophic (THB) and hydrocarbon utilizing bacteria (HUB) in the spent engine oil contaminated soil sample. The population of HUB was 3.9 x 10<sup>3</sup> cfu/g constituting 8.67% of the heterotrophic population of 4.5 x 10<sup>4</sup> cfu/g. The bacterial isolates identified in this study were spore forming Gram positive, motile rods, negative to indole and nitrate reduction tests, but showed positive reactions to urease, catalase, citrate tests. Plates 1 and 2 present the identity of SEO utilizing Bacillus based on the comparison morphological and biochemical characteristics

using the ABI software. The findings revealed the identity of SEO degraders as Bacillus niacini (90.4% similarity and 100% matrix integrity) and Bacillus circulans (87.9% similarity and 100% matrix integrity). The GC-MS profiles for the untreated spent engine oil (control) are presented in Table 3 and illustrated in Figure 1. In contrast, the chromatographic profiles of inoculated with Bacillus niacini and Bacillus circulans are shown in Figures 2 and 3, with corresponding compound data provided in Tables 4 and 5 respectively. The untreated control sample exhibited high molecular weight hydrocarbon compounds ranging from 270 to 354 g/mol. In comparison, samples treated with Bacillus niacini revealed a broader molecular weight distribution, ranging from 156 to 528 g/mol. Notably, a compound identified 1,3,4,6tetrabromopentacyclohexadecane, with molecular weight of 528 g/mol, was detected exclusively in the Bacillus niacini-treated sample. The elevated molecular weight is attributed to the presence of four bromine atoms in the molecule. Samples treated with B. circulans showed molecular weight ranging from 138 to 358 g/mol, suggesting further breakdown of complex hydrocarbons into smaller compounds. Overall, the GC-MS profiles indicated a general degradation of hydrocarbons in the treated samples, with noticeable shifts in compound composition, molecular weights, and retention times. This provides strong evidence of the biodegradation capabilities of both Bacillus niacini and Bacillus circulans.

Table 1: Population of bacterial types in the assayed spent engine oil contaminated soil

Bacterial type	Population (cfu/g)
THB	$4.5 \times 10^4$
HUB	$3.9 \times 10^3$
% HUB	8.67

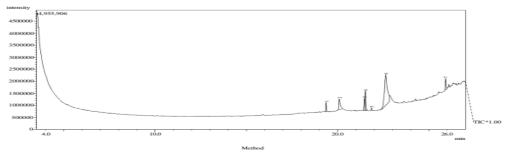


Figure 1: Gas Chromatography-Mass Spectrometry spectra of spent engine oil (control)

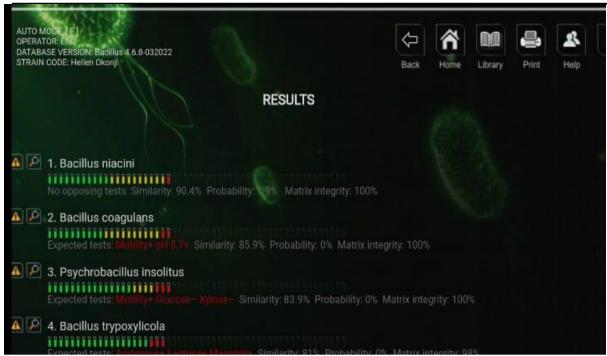


Plate 1: Database version of identified bacteria



Plate 2: Database version of the identified bacteria

Table 2: Gas Chromatography-Mass Spectrometry profile of compounds in spent engine oil (control)

	/					
Peak	R. T. (Mins)	Peak Area	Height	Compound Name	Compound Formula	Mol. Wt.
No		(%)	(%)			
1	19.364	3.87	9.98	Methyl-1, 4- methypentadecanoate	$C_{17}H_{34}O_2$	270
2	20.090	10.84	11.07	Octadecanoic acid	$C_{18}H_{36}O_2$	284
3	21.467	6.22	12.64	11,14-Eicosadienoic acid, methyl ester	$C_{21}H_{38}O_2$	322
4	21.514	10.68	20.39	Methyl 11 Octadecanoate	$C_{19}H_{36}O_2$	296
5	21.853	1.28	2.55	n-Docosanoic acid methyl ester	$C_{23}H_{46}O_2$	354
6	22.627	60.45	31.54	9,12-octadecadienoic acid	$C_{18}H_{32}O_2$	280
7	25.912	6.66	11.82	9,12-octadecadienoyl chloride	$C_{18}H_{31}ClO$	298

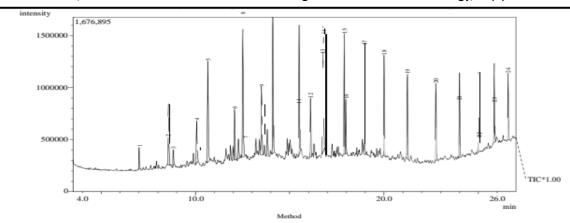


Figure 2: Gas Chromatography-Mass Spectrometry spectra of spent engine oil inoculated with *B. niacini* 

Table 3: Gas Chromatography-Mass Spectrometry profile of compounds in spent engine oil inoculated with *B. niacini* 

Peak	R. T.	Peak Area	Height	Compound Name	Compound	Mol.
No	(Mins)	(%)	(%)		Formula	Wt.
1	7.008	0.85	1.11	Hendecane	$C_{11}H_{24}$	156
2	8.570	3.48	3.17	Pilocarpine	$C_{11}H_{16}N_2O_2$	208
3	8.823	0.87	0.84	2,6-Dimethylundecane	$C_{13}H_{28}$	184
4	10.064	2.75	2.19	2,3,7-trimethyl octane	$C_{11}H_{24}$	156
5	10.657	6.09	5.22	Tridecane	$C_{13}H_{28}$	184
6	12.077	2.77	2.62	Hexadecane	$C_{16}H_{34}$	226
7	12.278	1.02	1.02	9, Octadecyne	$C_{18}H_{34}$	250
8	12.509	7.61	6.61	Hexadecane	$C_{16}H_{34}$	226
9	13.500	4.50	3.53	Hexadecane	$C_{16}H_{34}$	226
10	14.102	7.07	7.05	Hexadecane	$C_{16}H_{34}$	226
11	15.496	6.27	6.68	1,3,4,6-Tetrabromopentacyclohexadecane	$C_{16}H_{20}Br_4$	528
12	16.097	3.26	3.03	2,6,10-Trimethylpentadecane	$C_{18}H_{38}$	254
13	16.745	6.18	6.55	2-morpholinomethyl1,3-diphenyl-1,2-propanol	$C_{20}H_{25}NO_2$	311
14	16.790	5.28	5.89	2,6,10-Trimethylpentadecane	$C_{18}H_{38}$	254
15	17.890	5.45	6.26	Hexadecane	C <sub>16</sub> H34	226
16	17.974	2.61	2.84	2,6,10-Trimethylpentadecane	$C_{18}H_{38}$	254
17	18.955	5.10	5.74	Benzaldehyde, 3-phenoxy-, (4,6-dimethyl-1,3,5-triazin-2-yl) hydrazine	C16H15N7O	321
18	20.002	4.99	5.21	Hexadecane	C16H34	226
19	21.239	4.76	4.37	Eicosane	C20H42	282
20	22.749	4.72	3.99	Hexadecane	C16H34	226
21	24.007	4.25	4.39	1,3,3,6-Tetrabromopentacyclohexadecane	C16H20Br4	528
22	25.005	3.80	4.14	Eicosane	C20H42	282
23	25.843	3.24	4.10	2,4-Bis(2,2-dimethylpropyl)-1,3-diphenyl-1,3-	C28H40Si2	432
24	26.578	2.93	3.46	divinyl(1,3) disilethane Hexadecane	С16Н34	226

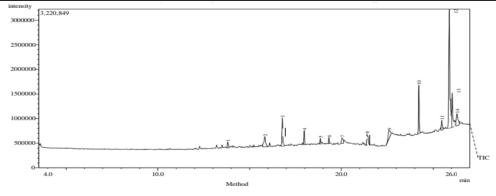


Figure 3: Gas Chromatography-Mass Spectrometry spectra of spent engine oil inoculated with *Bacillus circulans* 

Table 4: Gas Chromatography-Mass Spectrometry profile of compounds in spent engine oil inoculated with *Bacillus circulans* 

Peak No	R. T.	Peak	Area Height	Compound Name	Compound	Mol.
	(Mins)	(%)	(%)	-	Formula	Wt.
1	13.802	1.95	1.95	2,5,5-Trimethyl-1,6-heptadiene	$C_{10}H_{18}$	138
2	15.829	5.64	3.23	Phthalic acid,	$C_{20}H_{26}O_4$	330
3	16.784	6.44	8.82	Pentadecane	$C_{15}H_{32}$	212
4	17.974	2.86	4.51	Eicosane	$C_{20}H_{42}$	282
5	18.852	1.15	1.87	4,8-Dimethylundecane	$C_{13}H_{28}$	184
6	19.319	1.15	1.95	n-Docosanoic acid methyl ester	$C_{23}H_{46}O_2$	354
7	20.036	2.74	1.78	Nonadecanoic acid	$C_{19}H_{38}O_2$	298
8	21.397	2.58	3.44	9,12-Octadecadienoic acid, methyl	$C_{19}H_{34}O_2$	294
9	22.604	5.23	2.52	ester, 9-Octadecenal	C <sub>18</sub> H <sub>34</sub> O	266
-						
10	24.20	11.09	15.22	Hexadecanoic acid,2,3-dihydroxypropyl ester	C <sub>19</sub> H <sub>38</sub> O <sub>4</sub>	330
11	25.468	2.71	3.14	Isopropyllinoleate	$C_{21}H_{38}O_2$	322
12	25.881	40.12	37.08	9-octadecanal	C <sub>18</sub> H <sub>34</sub> O	266
13	26.039	10.08	10.64	Octadecyl vinyl ether	$C_{20}H_{40}O$	296
14	26.298	6.26	3.81	Octadecanoic acid, 2,3-	$C_{21}H_{42}O_4$	358
				dihydroxypropyl ester		

## **DISCUSSION**

In this study, the total population of total heterotrophic bacteria (THB) and total hydrocarbon utilizers (HUB) differ with the population of THB higher than HUB. The higher population of THB over HUB obtained in this study agrees with those reported in similar studies (Jesubunmi, 2014; Nyarko et al. 2020). The authors asserted that the higher population of THB relative to HUB is expected because HUBs are only a fraction of the total bacteria and part of the community. heterotrophic bacterial However, this finding was slightly similar to those obtained by Ayandele (2012) who recorded high bacterial counts which showed hydrocarbon-utilizing bacteria as high as  $35 \times 10^4$  to  $265 \times 10^4$  cfu/ml, and total bacteria counts from  $245 \times 10^4$  and 123 $\times 10^4$  cfu/ml.

Bacillus species have been variously isolated from different ecological habitat including oil contaminated and uncontaminated ecosystems. The *B. niacini* and *B. circulans* were among those isolated from previous studies (Yan et al., 2013; Eze et al., 2014). Eze et al. (2014) reported Bacillus species as hydrocarbon degraders from Umuahia, Abia State, Nigeria. Yan et al. (2013) reported the presence of mainly Bacillus spp. from oil contaminated soils in China. A study by Tirmizhi et al. (2022) also reported Bacillus

Pseudomonas as potential diesel and degrading bacteria. Also, a study by Ikuesan (2017; Ikuesan et al., 2020) reported the biodegradation potentials of some indigenous soil microorganisms isolated from crude oil contaminated soil. The isolation and identification of these bacterial isolates from the contaminated soil springs no surprise as Bacillus are soil borne microorganisms which are known to grow, multiply and increase in cell number during the degradation processes, thus. demonstrating abilities to utilize waste engine oil as sole source of carbon and energy (Enerijiofi et al., 2020).

The GC-MS spectra of the Bacillus niacini and Bacillus circulans inoculated medium indicated that the control comprises of aromatic and aliphatic esters. The molecular weights of compounds profiles from the findings ranges between 270 and 354 g/mol indicating high molecular weights compounds compared to sample treated with B. niacini and B. cirulans. The molecular weights of B. niacini treated sample ranges between 18 and 528, the high molecular weight observed in B. niacini treatment is due to the presence of four bromine atom in 1,3,4,6-tetrabromopentacyclohexadecane compound. Bromine is known to cycle naturally in soils and sediments through both processes microbial abiotic and

2014), transformation (Zhai, indicating possible microbial-mediated transformations in this context. The molecular weight of Bacillus circulans ranges between 151 and 358. The treated samples were compared with the control samples. A substantive number of low molecular weight compounds were identified in the treated sample. These are evidence of biodegradation of the complex compounds and shows that B. niacini isolated can degrade the hydrocarbon compounds present in the spent oil. The GC-MS profiles of the degraded compounds included the following; capric acid methyl hexadecanoic acid. 9.12ester. octadecadienoyl chloride, methyl-11octadecenoate. docosanoic acid, methyl heneicosanoate, 14-methyl-8-hexadecyn-1cyclohexanebutanol, 2-9-tetradecenal, etc. and the available compounds in with microorganisms. The variation the chemical retention structure. time. percentage area (area %), and the molecular weights with respect to each sample were not unconnected with bacterial activity. The findings showed that the microorganisms employed can degrade the chemical components of the spent crude engine oil. Bacillus spp. involving in the degradation of spent crude oil are ubiquitous, widely distributed and isolated from the diverse environments, such as soil and water biodegradability ecosystems. Based on potential, the isolates can utilize oil products as a source of energy and carbon (Alnuaimi et al., 2020). A study by Elkemary et al. (2023) reported the ability of Enterobacter spp. to degrade chemical compounds in oilpolluted soils. The presence of polycyclic aromatic hydrocarbons (PAHs) in the oilpolluted soil environments can be a source of carbon and energy needed for microbial growth and replication (Alnuaimi et al., The slow-process of microbial degradation of each PAH usually occur to a complete bioremediation chemical compounds from the oil-polluted soils. This shows that the concentration of PAHs in the control samples gradually degraded into simpler aliphatic compounds

with the low molecular weights compounds such as alkanes, organic acids alcohols and so on. Some of the compounds that were degraded by microorganism employed, are isomers of the compounds found in the control with the same chemical formula, but different compound name and structure. The biodegradation process makes it easy for the bio degraders to easily utilize the low molecular weight compounds, since the higher molecular weight compounds required enzymatic modifications, which limit their easy biodegradation (Abbasian et al., 2015). Conversely, it has been reported that these biodegraders may not prefer to degrade the high molecular hydrocarbons until the low molecular weight counterparts have been completely degraded (Ediagbonya et al., 2022). Ikuesan (2025) stated that biodegradation process influenced among other factors by bioavailability of xenobiotics to microorganisms. Sajna et al. (2015) and Chebbi et al. (2017) reported that low bioavailability hydrocarbon of to microorganisms is a serious threat to the efficiency biodegradation of process. Therefore, the ability of the cell-free supernatant of B. niacini and B.cirulans to cause the degradation of spent engine oil in this study is suggestive that these Bacillus species produce enzvmes or other extracellular secondary metabolites that enhanced their biodegradation activity. This assertion is derived from Elenga-Wilson et al. (2021) that biodegradation of petroleum hydrocarbon is influenced by the production of biosurfactants. Hence, the findings from this study revealed that the Bacillus spp. could help in the utilization of both long and short chain compounds in spent oil as a source of carbon. A study by Elkemary et al. (2023) reported the degradation potential of Egyptian bacterial consortium for oil spill treatment using GC-MS for identification of degraded products. Furthermore, based on the tolerance of *Bacillus* spp. to survive in unfriendly environment, their isolation from the same source using oil-rich medium could be research of interest for future exploration and as an excellent degrader of spent engineoil contaminated soils.

## **CONCLUSION**

This study investigated the biodegradation abilities of the two Bacillus species isolated from spent engine oil contaminated soil samples. The GC-MS profiles the degradation rate of polycyclic aromatic hvdrocarbons (PAHs) and aliphatic compounds present in the spent engine oil by the selected bacterial isolates. At the end of the study, the findings revealed that isolates belonging bacterial to genus Bacillus, have the capacity to metabolize

## **REFERENCES**

- Abbasian F, Lockington R, Mallavarapu, M and Naidu R (2015) A comprehensive review of aliphatic hydrocarbon biodegradation by bacteria. *Applied Biochemistry and Biotechnology* 176(3):670–699. https://doi. org/10.1007/s12010-015-1603-5.
- Adeleye, A. O., Nkereuwem, M. E., Omokhudu, G. I., Amoo, A. O., Shiaka, G. P. and Yerima, M. B. (2018). Effect of microorganisms in the bioremediation of spent engine oil and petroleum related environmental pollution. *Journal of Applied Sciences and Environmental Management*, 22:157-167.
- Agarry, S. E. and Ogunleye, O (2012). Factorial designs application to study enhanced bioremediation of soil artificially contaminated with weathered Bonny light crude oil through biostimulation and bioaugmentation strategy. *Journal of Environmental Protection*. 2012 (3):748-759.
- Alnuaimi, M. T., Taher, T. A., Aljanabi, Z. Z. and Adel, M. M. (2020). High-resolution GC/MS study of biodegradation of crude oil by *Bacillus megaterium*. *Research on Crops*, 21: 650-657.

degrade high molecular and weight hydrocarbons in spent engine oil. The GC-MS profiles showed that aliphatic and polycyclic aromatic hydrocarbons present in spent engine oil were degraded by the bacteria isolates. The isolations of the hydrocarbon-degrading bacteria suggest that the bacterial isolates could be useful in bioremediation of hydrocarbon polluted sites especially of the auto-mechanic and the mechanic village where engine oil pollution is common. Also, the isolates perhaps could as well combine with another bacterial consortium for replication in other oil contaminated sites.

- Ayandele, A. A. (2012). Isolation, characterisation and biodegradation ability of bacteria isolated from soil contaminated with hydrocarbons. Doctoral Thesis. University of Ibadan. Page 1-209.
- Bahadure, S., Kalia, R. and Chavan, R (2013) Comparative study of bioremediation of hydrocarbon fuels *International Journal of Biotechnology and Bioengineering Research*, 4 (7):677-686.
- Boboye, B. E., Ikuesan F. A. and Olukunle O. F. (2023) Comparative efficiency of different biological techniques in the remediation of petroleum oil polluted soil. *International Research Journal of Environmental Sciences* 12(2), 13-20.
- Chebbi A., Elshikh M. Haque F., Ahmed S., Dobbin S., Marchant R., Sayadi S., Chamkha M. and Banat, IM. (2017) Rhamnolipids from Pseudomonas aeruginosa strain W10; as antibiofilm/antibiofouling products for metal protection, *Journal of Basic Microbiology*. 57 (5) 364–375.
- Cheesbrough, M. (2006). District Laboratory Practice in Tropical Countries. 2<sup>nd</sup> Edition. Cambridge University Press, Cambridge, UK., ISBN-13:9781139449298. 50: 165-176.
- Das and Chandran, P (2011). Microbial degradation of petroleum

- hydrocarbon contaminants: An overview. *Biotechnology Research International*, 2011:941810.
- Das, A., Das, N., Rajkumari, J., Pandey, P. and Piyush Pandey, P. **Exploring** the bioremediation potential of **Bacillus** spp. for mitigation sustainable of hvdrocarbon contaminants. Environmental **Sustainability** https://doi.org/10.1007/s42398-024-0309-9
- Ediagbonya, T. F., Oluwafemi, A. O., Ikuesan, F. A., Ogunjobi, J. A. and Odinaka, C. V. (2022). The accumulation of polycyclic aromatic hydrocarbons in snails and soil in Igbekebo, Ondo State, Nigeria. *Regional Studies in Marine Science*, 56:102625.
- Ekpenyong, M. G., Antai, S. P. (2007) Influence of pH on cadmium toxicity to *Bacillus* species (02 and 12) during biodegradation of crude oil. *International Journal of Biological Chemistry* 1(1):29-37.
- Elenga-Wilson P. S, Kayath C. A., Mokemiabeka, N. S., Nzaou, S. A. E., Nguimbi, E. and Ahombo, G. (2021) Profiling of indigenous biosurfactant-producing *Bacillus* isolates in the bioremediation of soil contaminated by petroleum products and Olive oil. *International Journal of Microbiology*, 2021, https://doi.org/10.1155/2021/956593
- Elkemary, A., Abouelkheir, S. AbdelHakim, M., Sabry, S. A. and Ghozlan, H. A. (2023). Potential Egyptian bacterial consortium for oil spill treatment: Α laboratory simulation. Case Studies in Chemical Environmental Engineering, and 7:100278.
- Enerijiofi, K. E., Ahonsi, C. O. and Ajao, E. K. (2020). Biodegradation potentials of waste engine oil by three bacterial isolates. *Journal of Applied Science*

- and Environmental Management, 24:483-487.
- Eze, V. C., Onwuakor, C. E. and Orok, F. E. (2014).Microbiological and physicochemical characteristics of with soil contaminated used petroleum products in Umuahia, Nigeria. Journal Abia State, Applied and Environmental Microbiology, 2:281-286.
- Ikuesan, F. A., Boboye, B. E. and Adetuyi, F. C. (2015) Cow dung as inoculum carrier for the degradation of crude oil *Sky Journal of Microbiology Research*, 3(4), pp. 047 054.
- Ikuesan, F. A. (2017). Evaluation of crude oil biodegradation potentials of some indigenous soil microorganisms. *Journal of Scientific Research and Reports* 13:1-9.
- Ikuesan, F. A., Olukunle, O. F., Oyelakin, O. O. and Boboye, B. E. (2020). Molecular characterization of crude oil degrading bacterial isolates from polluted soils and cow dung. International Journal of Environment. Agriculture and Biotechnology, 5:533-540.
- Ikuesan, F. A. Ehuwa, I. and Adeleke, B. S. (2022). Petroleum derivatives' toxicity: influence on the growth of soil nitrifying bacteria. *Dutse Journal of Pure and Applied Sciences*, 8: 44.54.
- Ikuesan, F. A. (2025) A study of oceansource biosurfactant-producing and petroleum hydrocarbon degrading bacterial resource of the Atlantic Ocean, Araromi Sea-Side, Ondo State, Nigeria. *Biotechnology Journal International*, 29(2): 45-58.
- Jesubunmi, C. O. (2014). Isolation of oil degrading microorganisms in spent engine oil contaminated soil. *Journal of Biology, Agriculture and Healthcare*, 4:191-195.
- Love, C. R., Arrington, E. C., Gosselin, K. M., Reddy, C. M., Van Mooy, B.A., Nelson, R.K. and Valentine, D. L., (2021). "Microbial production and

- consumption of hydrocarbons in the global ocean." *Nature microbiology*, 6(4):489-p498.
- Nurulhuda, K., Syahir H., Nur, A. Y. and Mohd, Y. A. S. (2018). Biodegradation of petroleum hydrocarbons by *Bacillus* spp.: A review. *Bioremediation Science and Technology Research*, 6 (2):14-21.
- Nyarko, H. D., Okpokwasili, G. C., Joel, O. F. and Galyuon, I. A. K. (2020). Isolation and characterization of carbonoclastic bacteria diversity in oil-contaminated soil in Cape Coast Metropolis, Ghana. *Microbiology Research Journal International*, 30:62-74.
- Odeyemi, O. (2014) Two centuries of oil and gas (1860-2060) Available: www.unversalacademicservice.org
- Sahal, S., Khaturia, S. and Joshi, N. (2023).

  Application of microbial enzymes in wastewater treatment. *Genomics approach to bioremediation:*Principles, tools, and emerging technologies, pp 209-227. Wiley Online Library.
- Saina, K. V., Sukumaran, R. Gottumukkala, L. D. and Pandey A. Crude oil biodegradation (2015)aided by biosurfactants Pseudozyma sp. NII 08165 or its culture broth, *Bioresource* Technology 191(2015): 133–139.
- Sorescu, I. and Stoica, C. (2021). Advanced Bacterial Identification Software, an original tool for phenotypic bacterial identification. *Romanian Biotechnological Letters*, 26(6): 3047-3953.
- Tirmizhi, M., Faggo, A. A. and Gulumbe, B. H. (2022). Species of *Pseudomonas* and *Bacillus* isolated from refined oil-contaminated soil showed the potential to efficiently degrade diesel. *Journal of Biochemistry, Microbiology and Biotechnology*, 10:72-75.
- Uba, B. O. and Ifeanyi, V.O. (2013). Comparative study on the rate of

- biodegradation of crude oil by bacteria and fungi isolates. *Asian Journal of Science and Technology*, 4:004-007.
- Yan, S., Wang, Q., Qu, L. and Li, C. (2013). Characterization of oil-degrading bacteria from oil-contaminated soil and activity of their enzymes. *Biotechnology and Biotechnological Equipment*, 27: 3932-3938.
- Zhai. H., Zhang. X., Zhu. X., Liu. J., Ji. M., (2014). Formation of brominated disinfection byproducts during chloramination of drinking water: New Polar Species and Overall Kinetics, *Environmental Science & Technology* 48(5).