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ARTICLE

Estimation of the biodegradability potential of bacterial isolated from a gas flare polluted soil in Ologbo settlement, Edo State, Nigeria

Michael Jimmy Emejulu[*1], Udochukwu Ugueri[2], Atuanya E. I[3], Osulu Emmanuel Ogochukwu[3], Chikezie Chidimma Claribel[4].

[1]Department of Environmental Management and Toxicology, University of Benin, Benin-City Edo State, Nigeria.

[2]Microbiology Department, University of Agriculture and Environmental Sciences Umuagwo, P.M.B. 1038, Owerri, Imo State.

[3]Microbiology Department, University of Benin, Benin-City Edo State.

[4]Anatomy Department, Federal University of Allied Health Sciences, Enugu, Enugu State.

★Corresponding author Email: rev.dr.ud@gmail.com

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Abstract

Gas flaring is the most common source of global warming, causing environmental pollution and ecological disturbances. This study evaluated the environmental impact of gas flaring and possible bacterial isolates that can be employed in the remediation of petroleum hydrocarbon polluted soil. Soil samples collected from Ologbo Settlement in Edo State, Nigeria were analyzed for their physicochemical and petroleum hydrocarbon parameters. The Shake flask biodegradation test was carried with screening of hydrocarbon degrading bacterial isolates which were characterized using the 16S RNA analysis technique. Across the six locations (40, 80, and 120, 160, 200 and 1000 m) sampled from 40m to 1000m and the control, it was observed that there was a progressive increase in the soil pH, moisture content and electricity conductivity. In the other hand, there was a gradual decrease in the soil temperature, total hydrocarbon content and total organic carbon. The total petroleum hydrocarbon (TPH), oil and grease and polycyclic aromatic hydrocarbon (PAH) content showed statistical significance ($p<0.05$) compare to the control which implies that distances from the flare sites significantly influence the hydrocarbon parameters of the soil. From the molecular characterization of the bacterial isolates, the four isolates were *Acinetobacter tandoii* strain BASG143, *Bacillus cereus* strain Ou9, *Bacillus subtilis* strain BS3902 and *Pseudomonas aeruginosa* strain KAVKOI. These results show that these bacteria strains were able to degrade hydrocarbon contaminants.

Keywords: Gas Flaring; Hydrocarbon Degrading Bacteria; Soil Contamination; Biotreatability.

1. Introduction

Gas flaring is a significant source of pollution, with various environmental, ecological, and health consequences. Petroleum hydrocarbon contaminants from gas flaring contribute to air pollution, soil

contamination, and health risks for local communities (Varjani *et al.*, 2017). Efforts to mitigate soot formation through improved flare efficiency, gas recovery, and alternative combustion technologies are essential for reducing the environmental and health impacts of gas flaring (Das & Dash, 2017). Gas flaring is the process of burning off excess natural gas produced during oil extraction or processing, often in oil fields or refineries where capturing the gas is not feasible or cost-effective. While gas flaring is intended to safely dispose of excess gas, it produces several harmful byproducts, including soot, polycyclic aromatic hydrocarbon (PAHs), carbon dioxide, nitrogen oxides, methane, volatile organic compounds (VOCs), sulfur oxide and other fine particulate matter (Ibrahim *et al.*, 2024). The release of these contaminants during gas flaring is an important environmental concern due to their impact on air quality, soil, and human health. Carbon dioxide and methane are greenhouse gases that contribute to global warming, sulfur oxide contributes to acid rain, nitrogen oxide and particulate matter can cause respiratory issues. Also, polycyclic aromatic hydrocarbons (PAHs) and volatile organic carbons (VOCs) can have negative impact on humans, animal and plants (Eze *et al.*, 2022).

Over time, hydrocarbon accumulation can lead to soil nutrient imbalances, harming crops and vegetation around the flare site (Odeu *et al.*, 1986) Petroleum hydrocarbon derivatives that settle on the ground can accumulate in soils, leading to soil contamination (Head *et al.*, 2006). This can have multiple effects, such as: Disruption of soil microbial communities (e.g., reducing biodiversity or killing beneficial soil bacteria). Alteration of soil structure, making it harder for water and nutrients to reach plant roots (Varjani, 2017). Plant intoxication with heavy metal and PAHs present in soot is possible. Soil bacteria are crucial for maintaining soil health, performing functions such as nutrient cycling, organic matter decomposition, and protecting against pathogens (Ito & Ugbomeh, 2017; Umeaku *et al.*, 2019). However, gas flaring can have detrimental effects on soil bacterial communities by altering the structure and diversity of soil bacterial communities and disrupt nitrogen fixation, organic matter decomposition, and carbon cycling (Dung *et al.*, 2008; Okoye *et al.*, 2024).

Figure 1. Gas flaring at Warri refinery, Delta State, Nigeria. Source: (Premium Times April, 2019).

Figure 2. Ologbo gas flaring in Edo State, Nigeria. Source: (The Guardian, June, 2024).

Bacterial biodegradation of petroleum hydrocarbon contaminated soil involves the process by which microorganisms; especially bacteria break down or transform complex organic compounds found in petroleum hydrocarbon (Osuji *et al.*, 2006; Odokuma & Dickson, 2004). An incomplete combustion of petroleum hydrocarbon generates carbon-based byproducts which include: soot, polycyclic aromatic hydrocarbons (PAHs), aliphatic hydrocarbons, and other toxic substances (Das & Chandran, 2011).

Various bacteria can degrade petroleum hydrocarbon and its components (Amadi & Akobundu, 2014). These bacteria can be naturally present in the soil or introduced through bioremediation strategies

(Al-Wasify & Hamed, 2014). Common microbial groups involved in biodegradation include *Pseudomonas* spp., *Bacillus* spp., *Mycobacterium* spp., and *Sphingomonas* spp. These bacteria possess specialized enzymes, such as oxidases, dehydrogenases, and dioxygenases, which enable them to break down complex organic compounds (Emejulu & Atuanya, 2022; Atuanya *et al.*, 2011). The Mechanisms of biodegradation involves oxidation of aromatic compounds, mineralization and co-metabolism. Some environmental factors that influence biodegradation includes: nutrient availability, oxygen levels, temperature and pH (Chikere & Ekwuabu, 2014; Okoye *et al.*, 2020).

Several studies have identified three major bioremediation Strategies which are bio stimulation, bioaugmentation and composting (Chikere *et al.*, 2019; Atlas, 1981). Over the years, biodegradation process has encountered several challenges and limitations which include: slow degradation rates due to chemical recalcitrancy, toxicity of degradation byproducts and soil physical properties such as texture, moisture content, and organic matter, can influence the success of bacterial biodegradation. Bacterial biodegradation of petroleum hydrocarbon contaminated soil is a promising and growing field of environmental science offering a biological solution to the problem of soot pollution (Das & Chandran, 2011). Molecular characterization of bacterial involved in the bio-treatability of soot contaminated soil will help to identify these hydrocarbon degrading bacterial species up to their strain level which will enhance and speed up bioremediation of soot contaminated sites within a shorter time frame with precision. This study therefore focuses on the molecular characterization of bacteria involved in bio-treatability of petroleum hydrocarbon contaminated soil in Ologbo Settlement, Edo State, Nigeria.

2. Materials and Methods

Study area

The study area is the Oredo flow station situated in Ologbo settlement, Ikpoba-Okha Local Government Area of Edo State between latitudes 60 3' 31.68" and 60 3' 57.6" North of the Equator and longitudes 50 34' 52.74" to 50 35' 26.88" East of Greenwich.

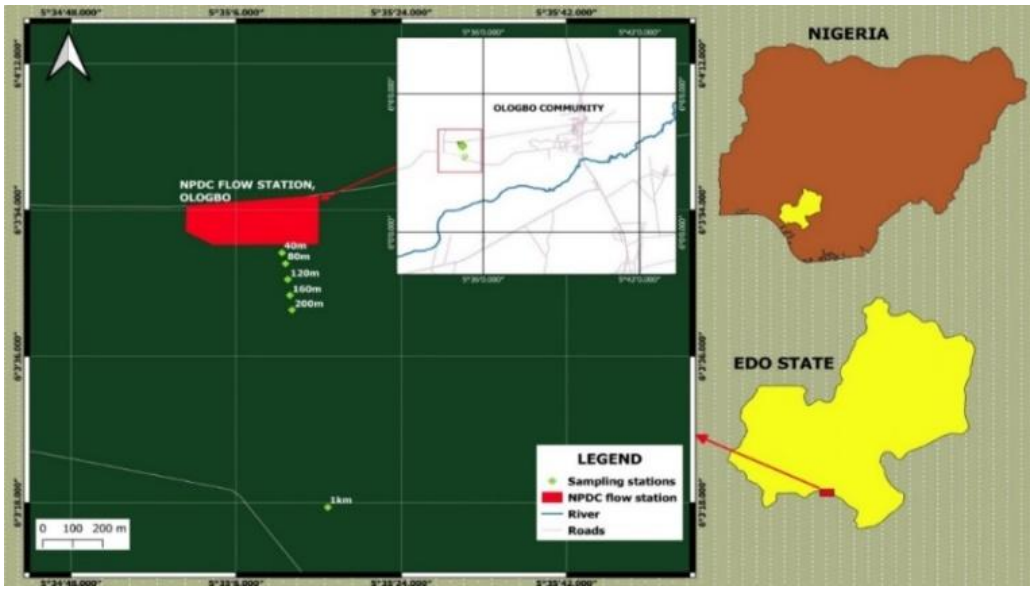

Figure 3. Map of Ologbo settlement, Edo State, Nigeria.

Soil sample collection

Top soil samples were collected within a 200 m radius from the flare stack at radial distances; 40m, 80m, 120m, 160m, 200m and 1km at the depths of (0-30 cm) using a standard soil auger. Soil samples were also

collected within the community where impact of the pollution is not experienced which served as the control soil sample. The soil samples were placed in sterile polyethylene bags, labeled accordingly and transported to the laboratory for analysis.

Soil Analysis

The soil samples were analyzed for the following parameters: pH, electrical conductivity, total organic carbon (TOC), total hydrocarbon content (THC), moisture content and essential minerals: Nitrate, Phosphorus, calcium, magnesium, sulphate, potassium and sodium (Udochukwu *et al.*, 2024).

Determination petroleum hydrocarbon parameters

The petroleum hydrocarbon parameters analyzed were, total petroleum hydrocarbon, oil and grease concentration and polycyclic aromatic hydrocarbons (PAHs). The total petroleum hydrocarbon was determined using ISO 16703 and gas chromatography (GC Perkin-Elmer/Clarias 500) with flame ionization detection. Total oil and greases concentration was determined using a spectrofluorimetric method as described by (Udochukwu *et al.*, 2020). A Gravimetric analysis was used to determine the total concentration of polycyclic aromatic hydrocarbons in the soil samples (Ezekoye *et al.*, 2018). Selected soil PAH was calculated using mean and standard deviation.

Molecular characterization of isolates

The *in situ* microbial community structure was monitored using 16S rDNA PCR random amplified polymorphic DNA (RAPD-PCR) technique. This was based on the extraction of DNA and resolving the diversity of the amplified sequence simply by differential electrophoretic migration on agarose gel (Hambali *et al.*, 2023).

Rapid-PCR

DNA was extracted using a Norgen DNA extraction kit. DNA (10–200ng), 200uM of each deoxynucleoside triphosphate (dNTP) (Promega), 2.0 mM MgCl2, IX PCR buffer, 50 pMol of primer, 2 units of Tap DNA polymerase (Promega), and sterile distilled water made up the reaction mix for PCR. Primer OPA-02(5'-TGC CGA GCT G -3') and OPC-04(5'CCG CAT CTA C 3') which are arbitrary primers were used (Ajiboye *et al.*, 2023). The PCR settings were 40 cycles of DNA chain extension, denaturation, and annealing (1 minute at 94 °C, 1 minute at 28 °C, and 2 minutes at 72 °C) in an Eppendorf master cycler gradient. Ethidium bromide staining was used to segregate the DNA fragments on a 1.5% agarose gel to make them visible. DNA molecular weight standards were 50bp DNA ladders (Bioline, USA). Digital cameras were used to take pictures of the gel (*Nwagwu et al., 2021).*

Screening test for the ability of selected isolates to utilize crude oil

The method employed in this analysis was adopted from (Ajiboye *et al.*, 2023). A loopful of bacteria from nutrient agar plates was suspended into 2 ml of mineral salt solution to create the cell suspensions. One set of control test tubes left un-inoculated, and the other control tubes were devoid of both glucose and crude oil. The mineral salt medium of volume 9.9ml was put in test tubes. To the set of 3 tubes, 0.1ml of crude oil was added. After autoclaving for 15 minutes at 121°C, the test tubes were cooled. Two drops of the bacterial suspension in sterile mineral salt broth were added to each set of tubes after they had cooled. After being cultured for 14 days at 28 ± 2°C, each tube was evaluated for turbidity (Nwagwu *et al.*, 2021).

Shake Flask biodegradation test

Organisms which showed the highest turbidity in the screening test were selected for the shake flask degradation experiment (Osuoha & Fakutiju, 2017). Four bacterial isolates were selected for this test. Crude oil from the same oil-bearing rock at gas flaring site were used as amendment, serving as the sole carbon and energy source for the study. The initial TPH concentration of the crude was determined. A modified mineral salt medium composed of $MgSO_4.7H_2O$, 0.42g/l, KCl, 0.30g/l, KH_2PO_4, 0.8g/l, K_2HPO_2, 1.3g/l,

$NaNO_3$, 0.42g/l (Ajiboye *et al.*, 2023) was used and the experiment was carried out in duplicate. This medium without crude oil amendment served as the control experiment. 150 ml of the medium and 10 ml of the crude were added to ten separate 250 ml conical flasks. To create the bacterial suspension for this experiment, a loopful of each isolate was suspended in 2 ml of mineral salt medium. Consortia of the bacterial inoculants were put into separate conical flasks, and each organism was added to a separate conical flask. All of the flasks were incubated for 28 days at room temperature with a rotary shaker running at 120 rpm. The pH, BOD, turbidity, and total viable counts were monitored on day 0, 7, 14, 21 and 28. Total petroleum hydrocarbon was also monitored at day 0 and day 28.

Determination of pH

The pH of the agitated flasks was evaluated at a 7 day interval for 28 days. The pH reading was obtained with the aid of a Hanna microprocessor pH meter which was earlier standardized with a 0.5M phosphate buffer.

Determination of the microbial counts

The mean viable bacterial count of the respective flasks was carried out at 7 day interval for 28 days. It was determined by the serial dilution and pour plate method (US-EPA, 2015). Nutrient Agar (NA) and peptone water were used as the diluents. Aseptic plating was carried out in triplicate. The Nutrient Agar plates were incubated at 30^{o}C for 48 hours.

Determination of Optical Density at 600 nm

A UV camp spectrophotometer was used to evaluate the optical density. A clean cuvette was filled with ten (10) milliliters of the sample, aseptically, and values of constant turbidity at 600 nm were taken. This was done at 7 days intervals for a period of 28 days (US-EPA, 2015).

Determination of biological oxygen demand (BOD)

The mineral salt medium containing the isolates in the biodegradation test was saturated with dissolved oxygen (DO) by aerating it with a supply of clean compressed air. To the distilled water, 1 ml of phosphate buffer solution (KH_2PO_4, K_2HPO_4, $Na_2HPO_4.7H_2O$, HN_4Cl), Magnesium sulphate solution and iron (iii) chloride solution was added. Dilutions of the prepared samples were then made to obtain adequate drop in oxygen content. Suitable amounts of the sample were measured into BOD bottles filled to the brim with the mineral salt medium. The initial dissolved oxygen (DO) was determined and after 5 days of incubation in the dark at 20^{o}C the final dissolved oxygen (DO_5) was determined using the formula below.

$$BOD_5\ (mg/l) = \frac{(DO_0 - DO_5) \times \text{volume of BOD Bottle}}{\text{ml of sample used}}$$

Where DO_0 = Dissolved oxygen found in the sample on the initial day (initial day may be termed day zero)
DO_5 = Dissolved oxygen found in the dilutions of the samples after titration on the final day.

Statistical analysis

All data were entered in Excel file (Microsoft Office 2016) and analyzed with GraphPad Prism version 8.0 for Windows software. All results were expressed as mean value ± standard error of the mean (S.E.M.). One-way ANOVA followed by post hoc Tukey's test was used to statistically compare the groups. Results were considered significant at $p < 0.05$

3. Results and Discussions

The results from this study showed a progressive increase in the soil pH, moisture content and electricity conductivity across the six locations (40, 80, 120, 160, 200 and 1000 m) sampled from 40 m to

1000 m and the control. In other hand, there was a gradual decrease in the soil temperature, total hydrocarbon content and total organic carbon. All the values recorded for soil pH, moisture content and electricity conductivity across the six locations at the different distances sample were all lower than the control while all the values recorded for soil temperature, total hydrocarbon content and total organic carbon across the six locations at the different distances sample were all higher than the control (Table 1). Soil pH had a peak value of 5.875±0.01 at 40m compared to the control with 7.035±0.02, moisture content recorded it highest value of 4.97±0.03 % still also at 40 m as against the control with 15.265±0.02 % and electrical conductivity also followed similar trend with 5.12±0.22 µs/cm at 40 m compared to the control with 46.24±0.08 µs/cm. The soil temperature was highest at 40m with 45.1±0.14ºC compared to the control with 31.15±0.07ºC, the total hydrocarbon content also had 5.18±0.08% at 40m compared to the control with 0.62±0.00%. The total organic carbon recorded its peak value of 5.41±0.27 % at 160 m compared to the control with 0.91±0.04 %. The physicochemical parameters of the flared-gas impacted soil water holding capacity, total organic nitrogen, nitrate, available phosphorus, cation exchange capacity, sand, silt and clay were all significant ($p<0.05$) compared to their controls. Their significance indicated that there was no variation from the control population.

Table 1: A comparison of the physicochemical parameters of the Gas flared impacted soil samples from the various points where the soil samples were collected and the control soil sample

	Distance from the gas flare stack (Meters)						
Parameters	**40**	**80**	**120**	**160**	**200**	**1000**	**Control**
Soil Temperature (^{0}C)	45.10±0.14	42.25±0.21	40.55±0.21	36.85±0.21	35.31±0.14	33.25±0.07	31.15±0.07
Soil Ph	5.88±0.01	6.02±0.01	6.71±0.02	6.82±0.01	6.90±0.02	6.91±0.01	7.04±0.02
EC, µs/cm	5.12±0.22	14.43±0.34	15.12±0.61	22.02±0.40	24.16±0.02	32.36±0.24	46.24±0.08
Moisture content, %	4.97±0.03	9.09±0.01	10.57±0.01	11.89±0.01	12.79±0.01	13.58±0.03	15.265±0.02
WHC, %	26.25±0.07	26.91±0.01	28.77±0.02	42.58±0.03	44.83±0.04	51.69±0.01	54.41±0.01
Bulk density, g/cm^3	0.82±0.03	0.785±0.01	0.765±0.01	0.75±0.00	0.71±0.01	0.7±0.00	0.68±0.00
TOC, %	1.89±0.01	1.93±0.01	3.15±0.07	5.41±0.27	1.15±0.07	0.97±0.02	0.91±0.04
THC, %	5.18±0.08	7.44±0.01	9.30±0.06	10.49±0.10	4.02±0.00	1.08±0.00	0.62±0.00
TON, %	0.08±0.00	0.11±0.00	0.19±0.01	0.21±0.01	0.27±0.01	0.29±0.01	0.54±0.03
Nitrate, mg/kg	9.99±0.01	11.12±0.18	11.85±0.04	11.86±0.03	12.38±0.2	12.78±0.06	13.86±0.08
Nitrite, mg/kg	0.57±0.01	0.58±0.00	0.74±0.00	0.78±0.00	0.86±0.00	0.9±0.00	0.98±0.00
Available Phosphorus, mg/kg	0.47±0.01	0.58±0.03	0.63±0.03	0.66±0.03	3.67±0.27	4.50±0.14	5.86±0.08
CEC, meq/100g	2.95±0.01	3.01±0.01	3.46±0.01	3.85±0.01	3.86±0	4.22±0.01	5.23±0.01
Potassium, mg/kg	1.90±0.14	1.40±0.14	0.60±0.14	0.65±0.07	1.45±0.07	0.70±0.00	1.45±0.07
Sand, %	98.04±0.20	97.24±0.01	96.88±0.30	96.11±0.50	95.24±0.20	87.64±0.10	81.43±0.10
Silt, %	1.38±0.01	1.71±0.02	2.12±0.51	2.88±0.04	3.45±0.21	9.52±0.21	13.33±0.57
Clay, %	0.52±0.01	0.87±0.02	1.00±0.15	1.01±0.03	1.30±0.25	2.84±0.11	4.84±0.01

Key: Electrical Conductivity (EC), Total Organic Carbon (TOC), Total Hydrocarbon Content (THC), Total Organic Nitrogen (TON), Water Holding Capacity (WHC), Cation Exchange Capacity (CEC)

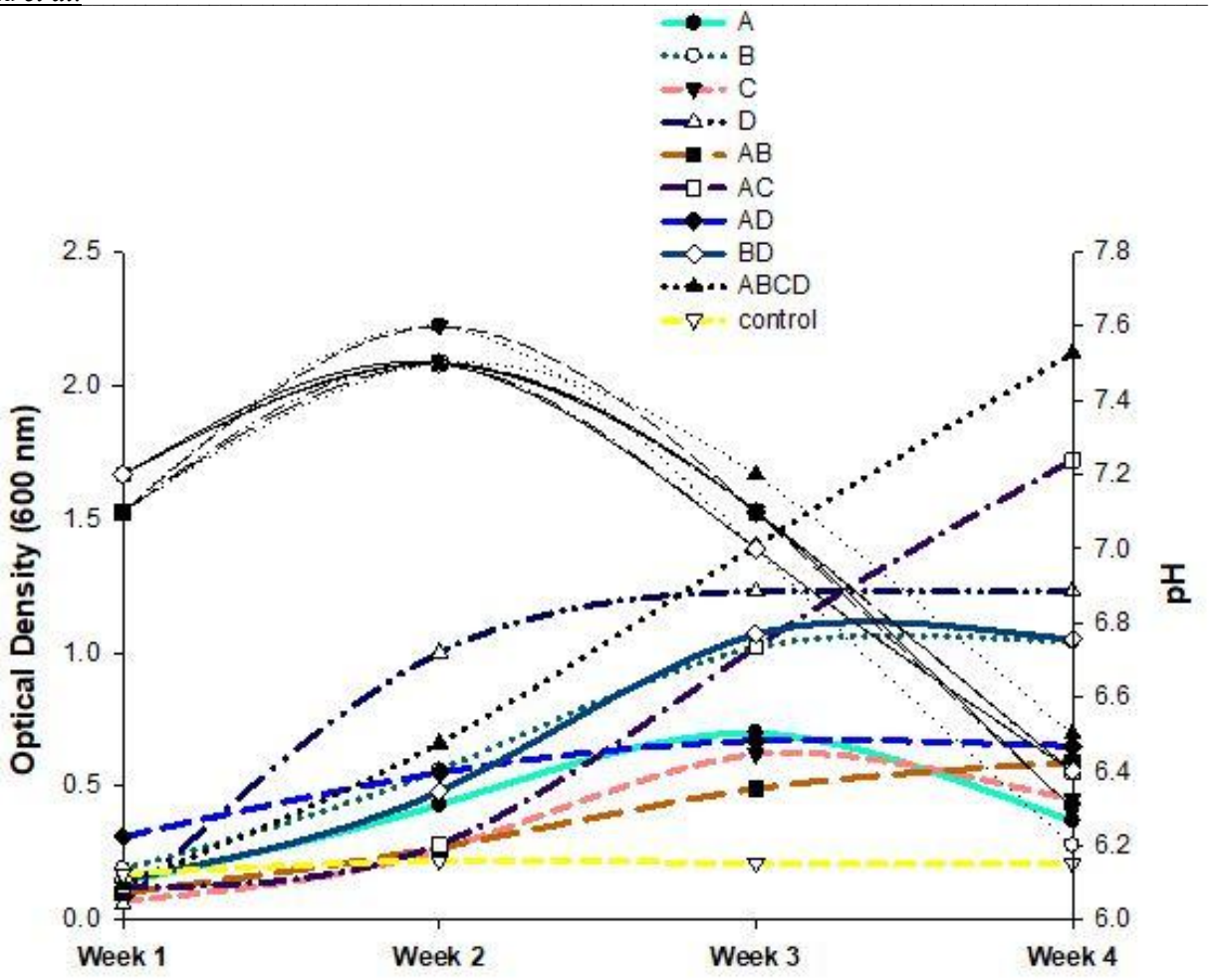

Figure 4. Bacteria growth curve after shake flask experiment.

A: *Acinetobacter tandoii*, **B**: *Bacillus cereus*, **C**: *Bacillus subtilis*, **D**: *Pseudomonas aeruginosa*, AB: *Acinetobacter tandoii* + *Bacillus cereus*, AC: *Acinetobacter tandoii* + *Bacillus subtilis*., AD: *Acinetobacter tandoii* +*Pseudomonas aeruginosa*., BD: *Bacillus cereus* + *Pseudomonas aeruginosa*., ABCD: *Acinetobacter tandoii* + *Bacillus cereus* + *Bacillus subtilis.* + *Pseudomonas aeruginosa*. Control: mineral salt medium

Table 2. A comparison of the petroleum hydrocarbon parameters of the Gas flared impacted soil samples from the various points where the soil samples were collected and the control soil sample

	Distance from the stack stalk (Meters)						
Organics	**40**	**80**	**120**	**160**	**200**	**1000**	**Control**
TPH (mg/kg)	123.1±1.10	253.1±0.39	311.2±6.90	475.01±0.02	411.4±08.40	115.1±19.40	18.3±3.16
Oil & grease content (mg/kg)	49.7±0.11	159.6±0.01	254.1±21.02	290.1±01.02	220.4±0.19	61.0±1.04	12.1±1.17
PAH (mg/kg)	1.10±0.01	1.21±0.09	1.45±0.02	1.72±0.06	2.00±1.01	0.90±0.04	0.10±0.01

Key: Total Petroleum Hydrocarbon (TPH), Poly Aromatic Hydrocarbon (PAH)

Table 3: Biodegradation parameters used to assess the degradation of crude oil by bacteria isolated from the TPH impacted soil

Time (days)	pH	BOD (mg/ml)	TOC (%)	Turbidity (ntu)
0	7.1	600	100	0.058
7	7.1	580	78	0.060
14	7.6	550	51	1.086
21	7.1	550	36	1.528
28	6.2	450	14	2.021

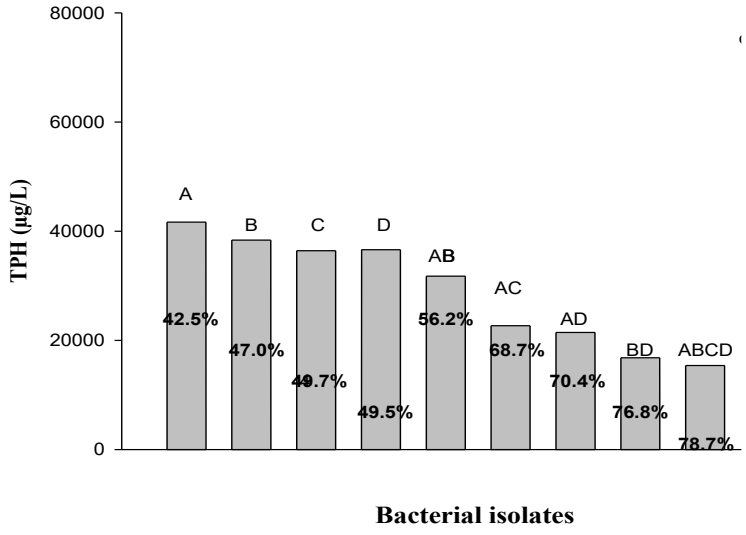

Figure 5. Comparative degradation of total petroleum hydrocarbon by different bacteria consortia.

Key: A: *Acinetobacter tandoii*, **B**: *Bacillus cereus*, **C**: *Bacillus subtilis*, **D**: *Pseudomonas aeruginosa*, AB: *Acinetobacter tandoii + Bacillus cereus*, AC: *Acinetobacter tandoii + Bacillus subtilis*, AD: *Acinetobacter tandoii +Pseudomonas aeruginosa*, BD: *Bacillus cereus+ Pseudomonas aeruginosa*, ABCD: *Acinetobacter tandoii + Bacillus cereus + Bacillus subtilis. + Pseudomonas aeruginosa*

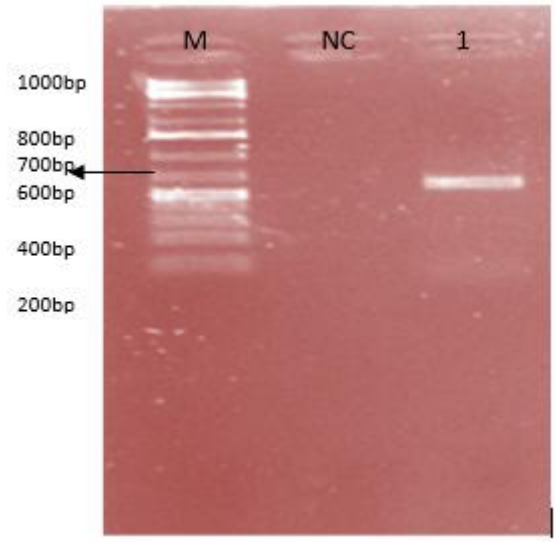

Figure 6. Electrophoresis result showing the presence of *Acinetobacter tandoii* with band at 700bp on lane 1.

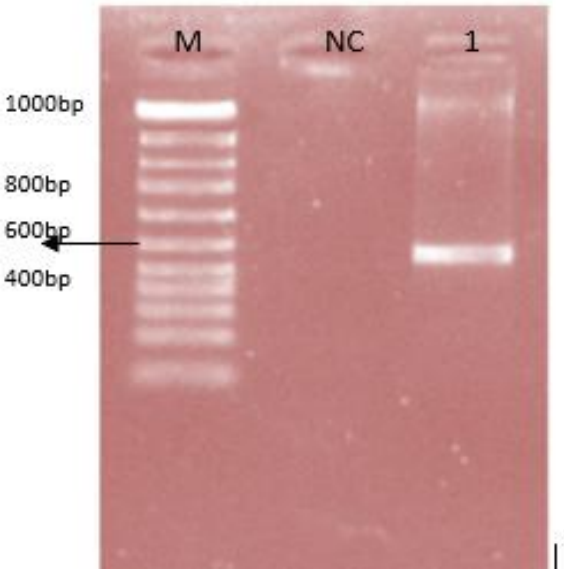

Figure 7. Electrophoresis result showing the presence of *Bacillus cereus* with band at 550bp on lane 1.

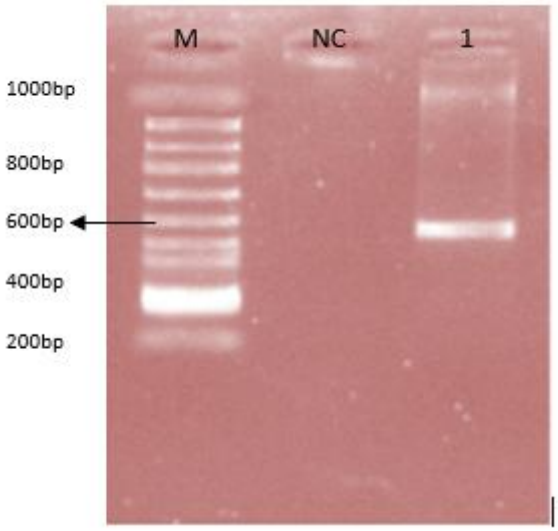

Figure 8. Electrophoresis result showing the presence of *Bacillus subtilis* with band at 600bp on lane 1.

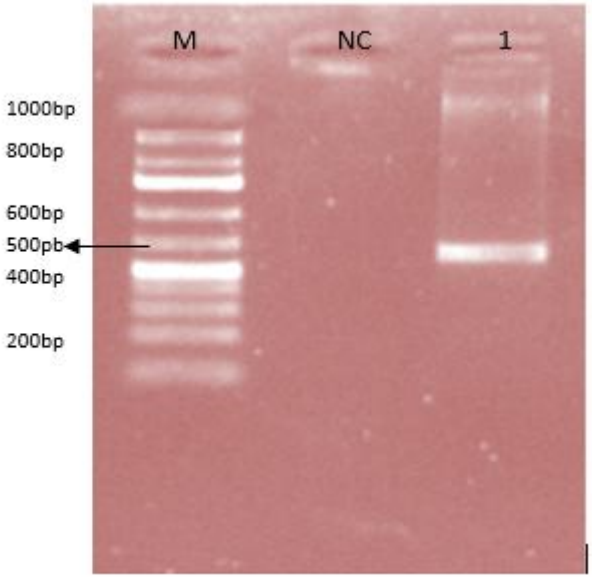

Figure 9. Electrophoresis result showing the presence of *Pseudomonas aeruginosa* with band at 500bp on lane 1.

Table 4. Suspected bacterial isolates showing their various percentage similarity and accession numbers

S/N	Sample Code	Accession No.	% Similarity	Suspected Isolates
1	TTA13	OQ780966.1	99.92	*Acinetobacter tandoii* strain BASG143
2	TTA15	KP128704.1	92.55	*Bacillus cereus* strain Ou9
3	TTD2	EU047884.1	100	*Bacillussubtilis* strain BS3902
4	TTA14	GQ865644.1	98.75	*Pseudomonas aeruginosa* strain KAVKOI

The 16S ribosomal RNA gene was used to further identify the isolates. The 16S rRNA gene universal primer set produced amplicons with 500 bp-sized fragments during PCR amplification. PCR were thereafter used as templates to run a bacterial species level, which generated PCR products of about 700 bp

for *Acienotobacter tandoii* (figure 6), 550 bp for *Bacillus cereus* (figure 7), 600 bp for *Bacillus subtilis* (figure 8) and 500 bp *Pseudomonas aeruginosa* (figure 9) in size as predicted for *Acienotobacter, Bacillus* and *Pseudomonas* sp. respectively. From the molecular characterization of the bacterial isolates, the four isolates were *Acinetobacter tandoii* strain BASG143, *Bacillus cereus* strain Ou9, *Bacillus subtilis* strain BS3902 and *Pseudomonas aeruginosa* strain KAVKOI. The nucleotide sequences obtained in this report were deposited with Genbank under the above accession numbers (Table 3).

The concentration of petroleum hydrocarbon parameters such as total petroleum hydrocarbon (TPH), Oil and grease content and Polyaromatic hydrocarbon (PAHs) content in the contaminate area is generally higher than the control area (Table 2). The TPH, oil and grease and PAH content showed statistical significance ($p<0.05$) compare to the control. This implies that distances from the flare sites significantly influence the hydrocarbon parameters of the soil (Ajiboye *et al.*, 2023; Ologunorisa, 2009). Their mean values of 1815.60 mg/kg and 1211.70mg/kg for TPH and Oil and grease respectively suggested a high petroleum hydrocarbon contaminated soil. The standard deviation values clearly show that the impact is diffuse and the dispersal of the hydrocarbon is extremely unequal over the whole region, with some places being more severely harmed than others. This outcome is consistent with the findings of Popoola & Olanbiwonninu. (2019).

The biodegradation test revealed growth in the mineral salt media which showed that some components of hydrocarbon were being degraded by the bacteria. The average turbidity result showed that there were growths recorded on the control sample with values ranging from 0.021 to 1.061 (ntu) and the crude oil sample had values ranging from 0.274 to 1.682 (ntu) which seems to be higher than that of the control sample (Table 3). The biochemical oxygen demand (BOD) and the percentage total organic carbon of the test system decreased gradually with time. There were four major dominant bacterial isolates which were culturally and morphologically identified to be *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Bacillus cereus* and *Acinetobacter tandoii.* The optical density and pH growth patterns of the bacterial isolates utilized in the shake flask degradation experiment are depicted in Figure 3. According to Al Wasify and Hamed (2014), the bacterial strains *Pseudomonas aeruginosa, Bacillus subtilis, Bacillus cereus,* and *Acinetobacter tandoii* employed in this investigation were among the most commonly isolated microorganisms from hydrocarbon-polluted areas which also agrees with Amadi & Akobundu, (2014). Among the four bacteria isolates, there were variations in the growth pattern and bacterial cell density. None of the bacterial isolates had lag periods, as seen by the growth patterns which could be attributed to either genetic make-up because hydrocarbon catalyzing enzymes are expressed constitutively or physiological because of prior exposure to exogenous hydrocarbons found in contaminated soils (Atuanya *et al.*, 2011; Okoye *et al.*, 2020). These bacteria used crude oil as their only source of carbon and energy, which led to their development and the concurrent creation of acid and consequently a drop in the pH of the system due to the presence of acidic metabolic byproducts (Nwagwu *et al.*, 2021; Chikere & Ekwuabu, 2014).

The findings of the present work showed that the degradation percentage increased as incubation increased and reaching its maximum after 28 days. The mixed bacterial consortia ABCD attained the highest level of biodegradation. These findings unmistakably demonstrated that after 28 days of incubation, the mixed bacterial culture was able to degrade crude oil at a highest rate of 78.7%, followed by the consortia BD, AD (76.8 %, 70.4.7 %, and 68.7 %, respectively).

Odokuma & Dickson (2004), had described the ability of mixed bacterial consortia to degrade 28–51% of saturates and 0–18% of aromatics hydrocarbon present in crude oil or up to 60 % crude oil, while in a study Fierer and Lennon. (2011), were a mixed bacterial consortium of *Micrococcus* sp*., Bacillus* sp., *Corynebacterium* sp*., Flavobacterium* sp*., and Pseudomonas* sp. carried out a maximum of 78 % of degradation for crude oil after 20 days of incubation while the maximum percentage of degradation by *Bacillus* sp*. and Micrococcus* sp. was 59 % and 49 %, respectively. Because no single strain of bacteria has the metabolic power to digest all the components contained in crude oil, the mixed bacterial culture produced the highest proportion of components that were degraded (Ujowundu, 2014). It is interesting to note that all of the species employed in this study are native to the habitat from which they were isolated, and all of those whose ability to breakdown organic pollutants was examined can do so actively.

The 16S ribosomal RNA gene was used to further identify the isolates. The 16S rRNA gene

universal primer set produced amplicons with 500 bp-sized fragments during PCR amplification. According to theoretical predictions made for the bacterial family (Das & Chandran, 2011; Ossai *et al.*, 2020), the findings of the present work are consistent with previous research. Amplicon from the first round of PCR were thereafter used as templates to run a bacterial species level, which generated PCR products of about 700bp for *Acienotobacter tandoii* (figure 6), 550 bp for *Bacillus cereus* (figure 7), 600 bp for *Bacillus subtilis* (figure 8) and 500 bp *Pseudomonas aeruginosa* (figure 9) in size as predicted for *Acienotobacter, Bacillus* and *Pseudomonas* sp. respectively. From the molecular characterization of the bacterial isolates, the four isolates were *Acinetobacter tandoii* strain BASG143, *Bacillus cereus* strain Ou9, *Bacillus subtilis* strain BS3902 and *Pseudomonas aeruginosa* strain KAVKOI. The nucleotide sequences obtained in this report were deposited with Genbank under the above accession numbers (Table 3). The result obtained in this study is in line with the previous studies (Udochukwu *et al.*, 2022). These results show that the strains are able to degrade hydrocarbon contaminants.

4.Conclusion

The most effective way to entirely eliminate oil pollution is by biodegradation of impurities. In order to provide a quicker method of cleaning up contaminants, biodegradation needs to be expedited. Microorganisms must maintain close physical contact with the material to be destroyed for bioremediation to be successful. Optimizing the development rate of native soil-degrading microflora is essential for enhancing the rate of biodegradation of contaminants. When all other options have been explored, the best way to optimize pollution cleanup is to increase the biodegradation rates of native microorganisms.

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Conflicts ofInterest

The authors declare no conflict of interest

Author Contributions

Conceptualization: JIMMY, E.M.; **Data curation:** UGUERI, U.; **Formal analysis:** UGUERI, U.; **Investigation:** JIMMY, E.M., UGUERI, U., OGOCHUKWU, O, E.; **Methodology:** JIMMY, E.M., UGUERI, U., OGOCHUKWU, O, E.; **Software:** JIMMY, E.M., UGUERI, U., OGOCHUKWU, O, E.; **Supervision:** ATUANYA, E. I..; **Validation:** JIMMY, E.M., UGUERI, U., OGOCHUKWU, O, E.; **Visualization:** JIMMY, E.M., UGUERI, U., OGOCHUKWU, O, E.; **Funding Acquisition:** CLARIBEL, C.C.; **Writing - original draft:** OGOCHUKWU, O, E. **Writing - review and editing:** OGOCHUKWU, O, E.

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