

Biore Restoration of Crude Oil-Polluted Sediment in Bodo Community Ogoniland, Nigeria Using Indigenous Bacterial Endophytes

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Abstract—High levels of petroleum-derived hydrocarbons present in crude oil-polluted sediments pose significant threats to living organisms in the environment due to their toxic, carcinogenic and mutagenic effects. In this study the laboratory scale bio-restoration of abandoned crude oil polluted mangrove sediment in Bodo community in Ogoniland, Nigeria using indigenous bacterial endophytes isolated from water hyacinth (*Eichhorniacrassipes*) was carried out. The indigenous bacterial endophytes were isolated, screened and used for bacterial consortium development. The bacterial consortium were identified using 16S illumina amplicon sequencing and applied in laboratory scale bioremediation for 42 days. The samples were monitored to confirm hydrocarbon biodegradation using the gas chromatography mass spectrometry (GC-FID). The bacterial population revealed that total culturable heterotrophic bacterial count (TCHBC) ranged from $1.56 \times 10^5 \pm 1.68$ CFU/g to $6.3 \times 10^6 \pm 2.35$ CFU/g while the total culturable hydrocarbon utilizing bacterial count (TCHUBC) ranged from $4.3 \times 10^4 \pm 9.12$ CFU/g to $1.69 \times 10^6 \pm 6.31$ CFU/g. There were variations in the abundance and diversity of the bacterial community present in the bacterial consortium which had huge biodegradation potential as revealed during the treatment/bioremediation process. The highest number of hydrocarbon-degrading bacteria from phylum to genus levels were Proteobacteria-Gammaproteobacteria-Pseudomonadales-Moraxellaceae-Acinetobacter, while the top 4 phyla identified are Proteobacteria, Bacteroidetes, Firmicutes, and Unclass_Bacteria. The highest net percentage loss of TPH and PAH were 93.17% and 94.34% respectively on day 42 in sample C while the lowest percentage TPH and PAH loss were 8.40% and 10.24% respectively on day 42 in sample A. Overall, this study has established the diversity and degradative ability of endophytic bacterial communities as bio-resource for the effective bio-restoration of crude oil-polluted environments.

Keywords—Endophytes, Hydrocarbon, Bioremediation, Ogoniland, Bacterial community, Biodegradation.

I. INTRODUCTION

Crude oil exploration and exploitation remains the most critical environmental issue in the Niger Delta region of Nigeria especially in Ogoniland Rivers State. Practically every sector of the Nigeria economy depends on the petroleum industry's goods and services either directly or indirectly, it has a significant multiplier effect on global economic growth (Wei and Guo, 2016). Bodo community in Ogoniland Nigeria being host to several crude oil facilities has experienced repeated oil spills for more than two decades. This has led to severe contamination of sediment, mangrove degradation, soil contamination, loss of aquatic life and even the loss of human lives. However, problems with environmental degradation, particularly from oil spills, can have a detrimental impact on the Nigeria economy (Lee *et al.*, 2015). The threat that sediment contamination poses to both human health and natural ecosystems, however, has made it a crucial global problem. Above a certain point, the mangrove sediment may completely lose its capacity to recover from such disturbances, resulting in long-term ecological, aquatic and health hazards. The presence of petroleum-derived hydrocarbon in the environment poses significant threats to living organisms present in such ecosystem because of its toxic, carcinogenic and mutagenic effects (Takaova *et al.*, 2013). Also, hydrocarbon presence can cause a shift in the microbial community composition of the affected environment (Maduwuba *et al.*, 2021).

In recent times, bioremediation using microorganisms has become visible as an eco-friendly option for the restoration of oil-polluted environment. Indigenous bacterial endophytes which are natural inhabitants of plant tissues in the mangrove ecosystem have been observed to exhibit high hydrocarbon-degrading ability which offer promising solution to environmental pollution. These endophytic bacteria are well-adapted to their indigenous environmental conditions of hydrocarbon pollution and can enhance hydrocarbon degradation while improving the native mangrove vegetation. Bioremediation is considered a realistic and low-cost alternative for treating extensive areas of pollution by organic chemicals. This technology can extract, remove, transform, or mineralize petroleum pollutants in a contaminated environment, transforming them into a less damaging, harmless, and stable form.

Endophytic bacteria are endosymbiotic organisms that occupy the area between plant cell walls and the xylem vessels in the roots, stems, and leaves to defend the plants against pathogens that can harm them (Fadiji and Babalola, 2020). Endophytes can be isolated using the traditional culture-dependent method on microbial or plant growth medium. The most frequent method used to detect and enumerate endophytes involves isolation from surface-sterilized host plant tissue. Endophytic bacteria have been shown to benefit plant establishment and survival in highly polluted soils by increasing nutrient uptake, enhancing plant tolerance to pollutants and degrading pollutants in plant tissues, which affects plant enzyme activity and secretes hormones,

siderophores, and other organic compounds (Wen *et al.*, 2011). Studies have revealed that endophytic bacteria that interact with their host plants can effectively speed up hydrocarbon degradation processes hence leading to environmental restoration (Oliveira *et al.*, 2012).

This study therefore investigates the hydrocarbon degrading ability of indigenous bacterial endophytes isolated from water hyacinth (*Eichhornia crassipes*) in a mangrove vegetation in Bodo community Ogoniland Nigeria for the bioremediation of crude oil-polluted sediment. By characterizing the endophytic bacterial community, monitoring their hydrocarbon-degrading ability, and applying the bacterial endophytes to access their effectiveness in improving sediment quality, this study aims to contribute to the development of eco-friendly, community-driven remediation approach of restoring Bodo community mangrove ecosystem and also support environmental recovery and sustainability.

II. MATERIALS AND METHODS

Site Description and sample collection

Sediment samples were collected from a crude oil-polluted mangrove swamp where oil industry operations have been halted in Bodo community Ogoni Land, River State, Nigeria. The water hyacinth (*Eichhornia crassipes*) were uprooted alongside the sediments from their roots using an Eckman grab, made into composite samples (homogenous mixture), and then put in sterile black polyethylene bags. All the samples were then moved to the laboratory at 4°C in an ice chest. The global positioning system (GPS) was used to compute each sample site co-ordinate. The co-ordinates are: point 1: 306867.00mE 508797.00mN; point 2: 306902.00mE 508911.00mN; point 3: 306867.00mE 508908.00mN; point 4: 306843.00mE 508833.00mN; point 5: 306878.00mE 508870.00mN.

Microbiological Analysis of Samples

The samples were analyzed for total culturable heterotrophic bacteria and total culturable hydrocarbon-utilizing bacteria.

1g of the sediment samples, where appropriate, were measured and put into 9 ml sterile normal saline as a diluent (0.85 % NaCl w/v in distilled water) in glass test tubes. The samples were corked and swirled to mix properly. This represented 10^{-1} dilution. Using a new pipette in each case, 1ml of the homogenate was transferred into another test tube containing 9ml sterile diluents to make 10^{-2} dilutions. The procedure was repeated to achieve the dilution of up to 10^{-6} for each of the samples analyzed. The total culturable heterotrophic bacteria (TCHB) was carried out using nutrient agar (Accumedia, Sweden) while total culturable hydrocarbon-utilizing bacteria (TCHUB) was done using the Bushnell Haas Agar amended with 1 % v/v Bonny light crude oil and 0.01 % w/v nystatin. All media were prepared following the manufacturer's instructions. A volume of 100 μ L each of 10^{-3} – 10^{-6} dilutions of the individual samples was spread onto the corresponding medium. The inoculated plates were then incubated at 30 °C for 24 h for TCHB and 96 hrs for

TCHUB (APHA, 2005). The total viable cells were estimated in Cfug.

Isolation and enrichment of hydrocarbon utilizing bacteria (HUB)

The isolation of hydrocarbon-utilizing bacteria in the samples was carried out using the method of Mnif *et al.* (2009) and Maduwuba and Dike (2024) with slight adjustments. 10g for the soil sample and 10 mL for the water samples, respectively were mixed with 100 ml of normal saline in a 250 mL Erlenmeyer's flask (Pyrex, USA). The mixture was vortexed for 2 minutes at high speed and then allowed to settle for about 5 minutes. 5ml of the supernatant was inoculated into a separate conical flask that contained 100ml Bushnell Haas Broth (BHB) (containing in g/L: 0.42MgSO₄·7H₂O, 0.02CaCl₂·2H₂O; 1KH₂PO₄; 1K₂HPO₄; 1 NH₄NO₃; 0.05FeCl₃; 15g agar powder, 0.1g nystatin) supplemented with 1% v/v Bonny light crude oil. Tween 80 (0.05 % v/v) was introduced to improve hydrocarbon degradation. The process was replicated for each sample in triplicate and then incubated in a shaker for 7 days at 150 rpm. The resulting colonies were sub-cultured into a fresh BHB medium supplemented with 1% v/v crude oil after which 1ml of the broth was diluted appropriately to make 10^{-1} – 10^{-6} dilutions and aliquots inoculated on Bushnell Haas agar (Wedulo *et al.*, 2014). Discrete colonies were achieved using the standard streaking method on the appropriate medium.

Different colonies were picked using a sterile inoculating wire loop and sub-cultured for purification to obtain discrete colonies. The pure strains were later sub-cultured on nutrient agar and then stored in Bushnell Haas agar slants supplemented with 1 % v/v crude oil. Morphological characteristics of individual colonies were recorded. Gram staining and different biochemical tests were performed on the resulting pure isolates.

Degradation screening test

Hydrocarbon utilization by the bacterial isolates was screened using the turbidimetric method. Turbidity was assessed using a UV-5200 spectrophotometer (Shanghai metash, China). A volume of 50 mL Bushnell Haas broth supplemented with crude oil 1 % (v/v) and 0.05 % Tween 80 was prepared and dispensed into Erlenmeyer's flasks and sterilized in an autoclave at 121 °C (15 psi) for 15 minutes. After sterilization, the broth was inoculated with 5 % (v/v) of the standardized inoculum of the test bacterial isolate. The inoculated flask containing the test bacterial isolates was incubated for 15 days in a shaker incubator at 150 rpm. The turbidity of the broths was measured using a UV-5200 spectrophotometer (Shanghai metash, China) every five days throughout the incubation period. Degradation was scored every three days based on the turbidity of the broth and oil emulsification in the medium.

Source of Nutrient Supplements and Bioremediation Experimental Setup

The cow dung was collected from a commercial livestock farm, while the inorganic fertilizer (NPK20:20:20) was produced by the NOTORE fertilizer company and collected

from the Ministry of Agriculture Port Harcourt Rivers State, Nigeria.

The experimental setup consists of four (4) duplicate experimental units. The setups were monitored for 42 days. Qualitative data that emerged in the treatments during the study were collected and compared for each unit. The details of the unit are stated in the table below;

Laboratory Bioremediation / Treatment Setup

A: 2000g polluted sediment only (negative control)
B: 2000g polluted sediment + 200 ml consortium (test treatment)
C: 2000g polluted sediment + 200 g NPK (positive control I)
D: 2000g polluted sediment + 200 g cow dung (positive control II)

16S rRNA Amplicon Sequencing

The method, according to Maduwuba and Ubani (2024) was adopted. 150 mg of each sample was aseptically measured and mixed with 2 mL of the nucleic acid preservation buffer. Each sample's bacterial DNA extraction was done using Laragen's validated proprietary bacterial DNA extraction protocol (Caporaso *et al.*, 2012).

The 16S V4 libraries were generated using methods adapted from (Minich *et al.* 2018) from the Earth Microbiome Project. The protocol comprises a miniaturized (5- μ L volume), high-throughput (384-sample) amplicon library preparation and the Echo 550 acoustic liquid handler. The samples were concentrated by the addition of 5–10% PhiX (Caporaso *et al.*, 2012).

The primer sets used for amplification were adapted based on the original 515F–806R primer pair modification (Caporaso *et al.*, 2012). The primer sequences are as stated below as:

Updated sequences: 515F – 806R forward-barcoded;
Forward: GTGYCAGCMGCCGCGGTAA; Reverse: GGACTACNVGGGTWTCTAAT
Original sequences: 515F – 806R reverse-barcoded;
Forward: GTGCCAGCMGCCGCGGTAA; Reverse: GGACTACHVGGGTWTCTAAT

In 25- μ L PCR reactions, amplifications were done in triplicate. The PCR reactions for the samples were pooled together into a single volume (75 μ L). Care was taken to avoid combining amplicons from different samples. Amplicons were run on an agarose gel with a ~300–350 bp band size. A bioanalyzer was used to detect the presence of PCR products for samples with no visible or faint bands. Amplicons were quantified using Quant-iT PicoGreen DNA assay kit (ThermoFisher/Invitrogen cat. no. P11496) following the instructions from the manufacturer. Twenty-four nanograms of each amplicon were combined in a single, sterile tube. Larger quantities were also used as long as the final pool can be isolated using the gel. Amplicon pool was cleaned using MoBio UltraClean PCR clean-up kit (cat. no. 12500) following the manufacturer's directives. The A260:A280 ratio of the cleaned final pool was determined by measuring the amplicon concentrations. The A260:A280 ratio is typically between 1.8 and 2.0 for optimal outcomes.

The Ribosomal Database Project (RDP) classifier was used to assign sequences derived from bacterial 16S genes to the corresponding taxonomic model (McMurdie and Holmes, 2012).

The phyloseq package in the R/Bioconductor package was used. Phyloseq objects containing OTU tables were created from the classifier output in hier format. Thereafter, sample data containing the sample's information were added to the phyloseq object for the downstream analysis. Other R packages implemented in this analysis includes ggplot2, GUniFrac, phangom, vegan and iNEXT.

Physio-chemical analysis of samples

pH was determined according to the procedure described by Maduwuba *et al.* (2021). Total petroleum hydrocarbons (TPH) and polycyclic aromatic hydrocarbons (PAH) residues were extracted from the samples and measured with the gas chromatography-flame ionization detector (GC-FID) system HP5890 series II, USA.

III. RESULTS

The total culturable heterotrophic bacterial counts (TCHBC) during treatment revealed that sample A recorded a TCHBC of $1.56 \times 10^5 \pm 1.68$ cfu/g on day 1, $2.3 \times 10^6 \pm 8.72$ cfu/g on day 14, $6.1 \times 10^6 \pm 5.28$ cfu/g on day 28, and $3.1 \times 10^5 \pm 6.91$ cfu/g on day 42. Sample B recorded a TCHBC of $2.6 \times 10^5 \pm 2.11$ cfu/g on day 1, $3.1 \times 10^6 \pm 6.62$ cfu/g on day 14, $4.2 \times 10^6 \pm 7.20$ cfu/g on day 28, and $2.2 \times 10^6 \pm 3.86$ cfu/g on day 42. Sample C recorded a TCHBC of $2.16 \times 10^5 \pm 4.01$ cfu/g on day 1, $4.6 \times 10^5 \pm 4.26$ cfu/g on day 14, $8.9 \times 10^5 \pm 7.80$ cfu/g on day 28, and $4.95 \times 10^5 \pm 3.41$ cfu/g on day 42. Sample D recorded TCHBC of $2.1 \times 10^6 \pm 1.88$ cfu/g on day 1, $4.9 \times 10^6 \pm 1.42$ cfu/g on day 14, $6.3 \times 10^6 \pm 2.35$ cfu/g on day 28, and $5.1 \times 10^6 \pm 3.52$ cfu/g on day 42. The highest TCHBC of $6.3 \times 10^6 \pm 2.35$ cfu/g was recorded in sample D on day 28 while the lowest TCHBC of $1.56 \times 10^5 \pm 1.68$ cfu/g was recorded in sample A on day 1. There was an overall increase in TCHBC from day 1 to day 28 then a significant decrease in TCHBC at day 42 for all the samples.

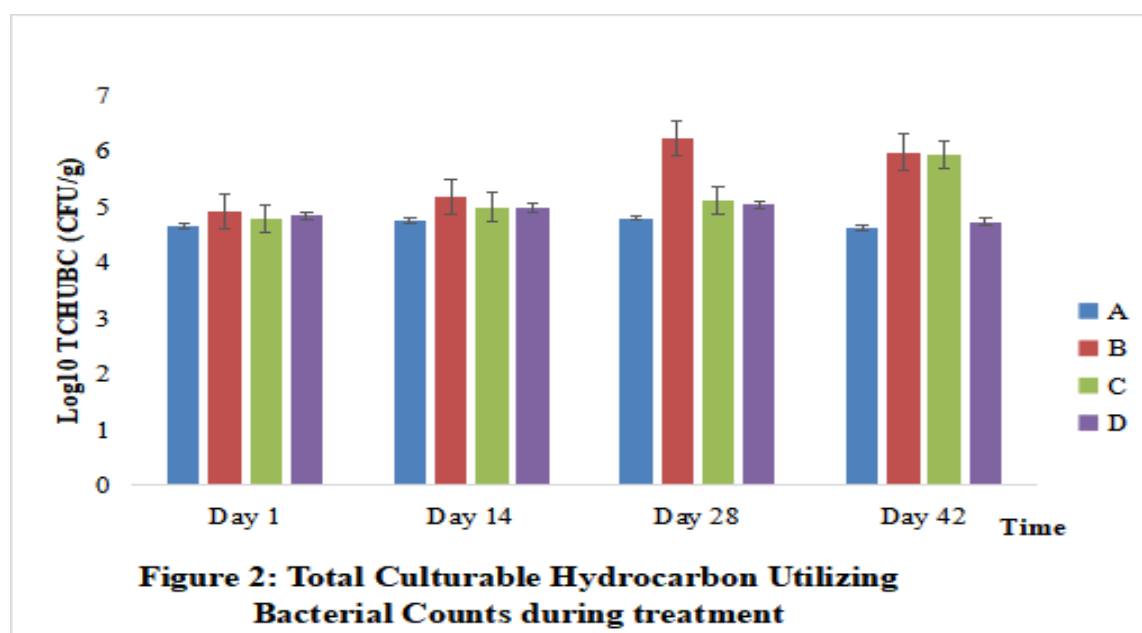
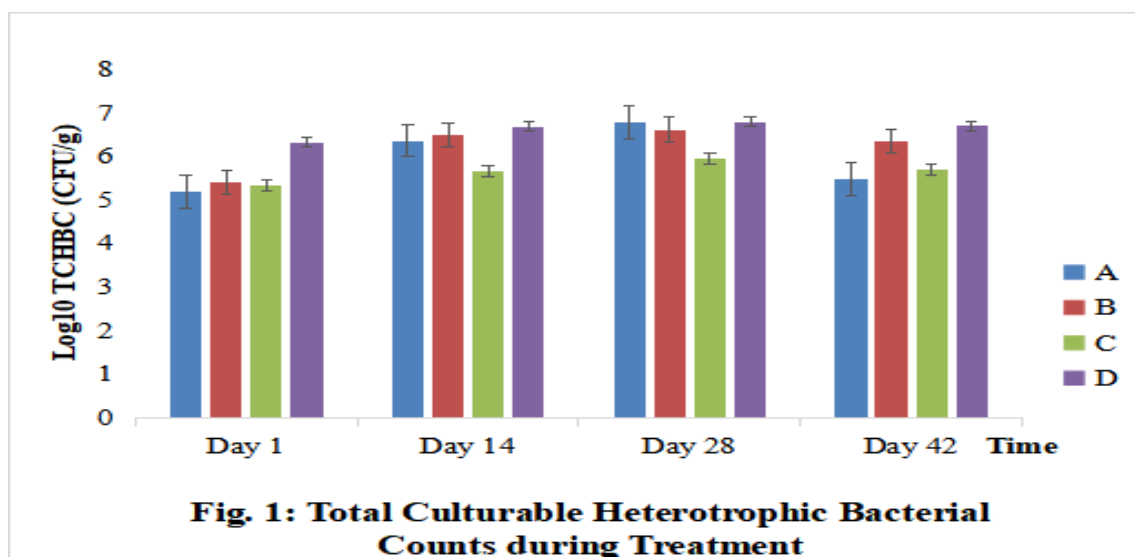
This is represented in figure 1.

The total culturable hydrocarbon utilizing bacterial counts (TCHUBC) during treatment revealed that sample A recorded a TCHUBC of $4.7 \times 10^4 \pm 8.27$ cfu/g on day 1, $5.9 \times 10^4 \pm 4.12$ cfu/g on day 14, $6.5 \times 10^4 \pm 6.25$ cfu/g on day 28, and $4.3 \times 10^4 \pm 9.12$ cfu/g on day 42. Sample B recorded a TCHUBC of $8.4 \times 10^4 \pm 7.11$ cfu/g on day 1, $1.52 \times 10^5 \pm 6.125$ cfu/g on day 14, $1.69 \times 10^6 \pm 6.31$ cfu/g on day 28, and $9.9 \times 10^5 \pm 2.80$ cfu/g on day 42. Sample C recorded a TCHUBC of $6.4 \times 10^4 \pm 9.25$ cfu/g on day 1, $1.03 \times 10^5 \pm 4.4$ cfu/g on day 14, $1.36 \times 10^5 \pm 3.80$ cfu/g on day 28, and $8.7 \times 10^5 \pm 4.33$ cfu/g on day 42 while Sample D recorded a TCHUBC of $7.1 \times 10^4 \pm 4.22$ cfu/g on day 1, $9.9 \times 10^4 \pm 3.83$ cfu/g on day 14, $1.11 \times 10^5 \pm 1.42$ cfu/g on day 28, and $5.5 \times 10^4 \pm 6.72$ cfu/g on day 42. The highest TCHUBC of $1.69 \times 10^6 \pm 6.31$ cfu/g was recorded in sample B on day 28 while the lowest TCHUBC of $4.7 \times 10^4 \pm 8.27$ cfu/g was recorded in sample A on day 1.

There was an overall increase in TCHUBC from day 1 to day 28 then a decrease in TCHUBC on day 42 except for

sample C which also recorded an increase in TCHUBC at day 42.

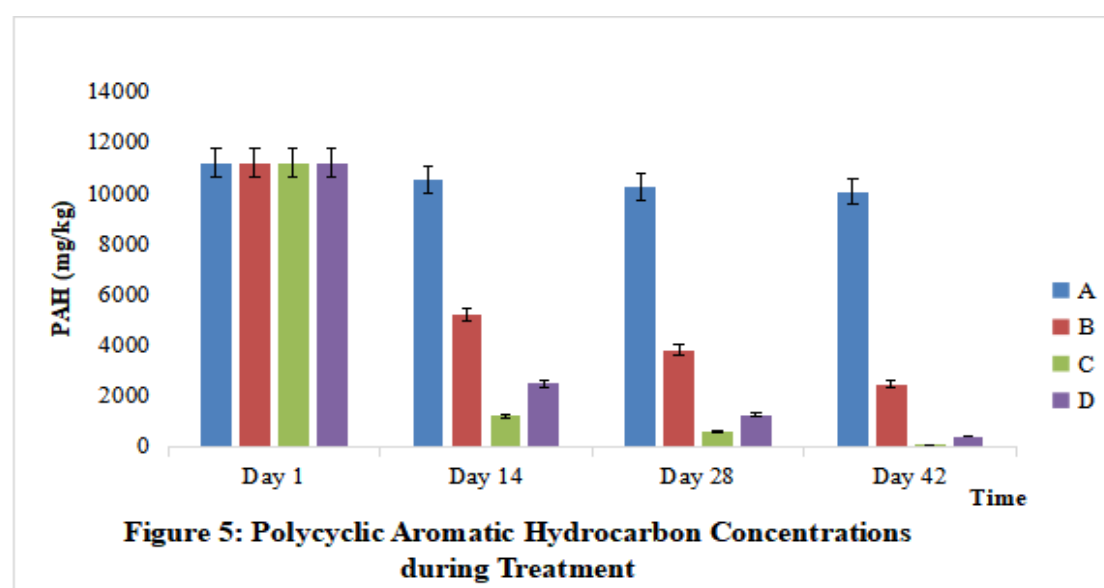
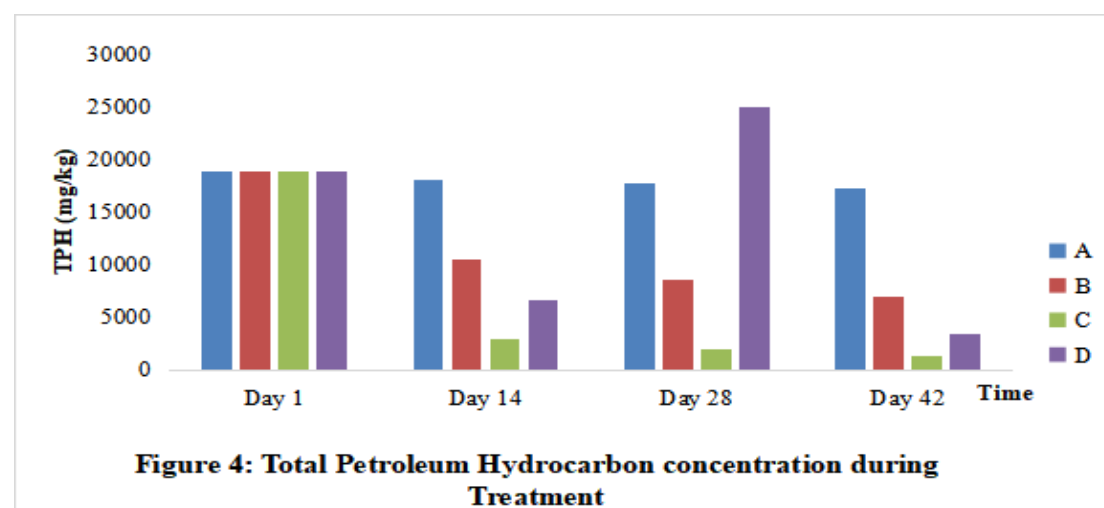
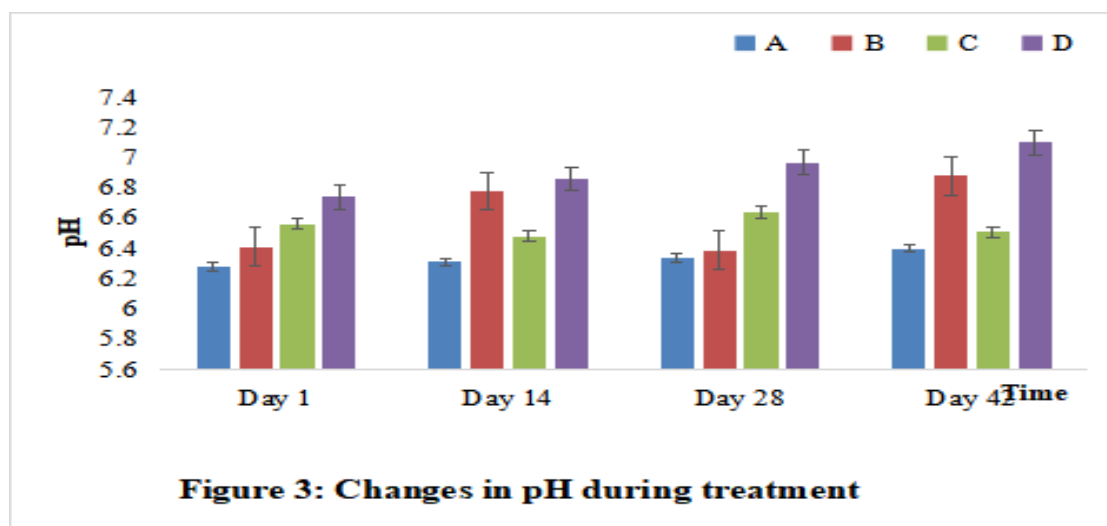
This is represented in figure 2.



The pH value of the individual treatment set up increased overtime from 6.28 ± 0.016 to 6.31 ± 0.014 , 6.34 ± 0.024 and 6.4 ± 0.037 at day 1, 14, 28 and 42 respectively in sample A. For sample B, pH value increased from 6.41 ± 0.02 at day 1 to 6.78 ± 0.176 at day 14 then decreased to 6.39 ± 0.179 at day 28 and increased to 6.88 ± 0.211 at day 42. In sample C, pH value changed from 6.56 ± 0.133 to 6.48 ± 0.126 to 6.64 ± 0.065 and 6.51 ± 0.069 at day 1, 14, 28 and 42 respectively. Also, for sample D, pH value changed from 6.74 ± 0.094 , to 6.86 ± 0.145 to 6.97 ± 0.93 and 7.10 ± 0.098 at day 1, 14, 28 and 42 respectively. The highest pH value was recorded in sample D at day 42 while the lowest pH value was recorded in sample A at day 1. This is represented in figure 3.

The gas chromatography analysis carried out revealed an overall reduction in TPH concentration throughout the

treatment period. Sample A recorded a TPH of 18945.04 ± 2.06 mg/kg at day 1, 18091.29 ± 3.69 mg/kg at day 14, 17800.34 ± 5.73 mg/kg at day 28 and 17353.39 ± 6.04 mg/kg at day 42 respectively. For sample B, TPH decreased from 18945.04 ± 2.06 mg/kg at day 1 to 10498.15 ± 4.14 mg/kg at day 14, 8561.83 ± 3.89 mg/kg at day 28 and 7025.51 ± 4.65 mg/kg at 42. Sample C revealed a change in TPH from 18945.04 ± 2.06 mg/kg a day 1 to 3011.23 ± 5.02 mg/kg, 2056.33 ± 6.01 mg/kg and 1299.42 ± 6.22 mg/kg at day 14, 28 and 42 respectively. Sample D revealed a change in TPH from 18945.04 ± 2.06 mg/kg at day 1 to 6589.167 ± 4.00 mg/kg, 5020.22 ± 6.21 mg/kg and 3485.28 ± 5.73 mg/kg at day 14, 28 and 42 respectively. The highest TPH concentration was recorded at day 1 for all samples while the lowest TPH concentration was recorded at day 42 in sample C. This is represented in figure 4.



The polycyclic aromatic hydrocarbon analysis revealed an overall reduction in the PAH concentration throughout the

treatment period. Sample A decreased from 11199.22 ± 1.98 mg/kg at day 1 to 10544.75 ± 7.21 mg/kg, 10258 ± 3.50 mg/kg

and 10052.74 ± 7.21 mg/kg at day 14, 28 and 42 respectively. For sample B, PAH decreased from 11199.22 ± 1.98 mg/kg at day 1 to 5183.32 ± 4.63 mg/kg, 3822.05 ± 2.89 mg/kg, and 2470.69 ± 6.11 mg/kg at day 14, 28 and 42 respectively. For sample C, PAH concentration reduced from 11199.22 ± 1.98 mg/kg at day 1 to 1215.805 ± 6.01 mg/kg, 604.71 ± 4.64 mg/kg, and 73.61 ± 3.0 mg/kg at day 14, 28 and 42 respectively. Also, for sample D, PAH concentration reduced from 11199.22 ± 1.98 mg/kg at day 1 to 2476.167 ± 4.42 mg/kg, 1241.851 ± 6.44 mg/kg and 407.535 ± 5.28 mg/kg at day 14, 28 and 42 respectively. This is represented in figure 5.

The major portion of the sequence of bacterial diversity in the consortium obtain at phylum level showed that the bacteria present were affiliated to top 4 phyla namely; *Proteobacteria* (96.12 %), *Firmicutes* (1.94 %), *Bacteroides* (0.45 %), and *Unclass_bacteria* (1.47 %). The dominant phylum is *Proteobacteria* with the highest relative abundance of 96.12 % while the phylum with the lowest relative abundance is *Bacteroides* (0.45 %) as shown in figure 6. The distribution of the phyla into different classes further revealed that they were shared into top 9 classes namely; *Betaproteobacteria* (52.53 %), *Gammaproteobacteria* (25.25 %), *Unclass Proteobacteria* (7.8 %), *Unclass Bacteria* (5.85 %), *Alphaproteobacteria* (3.9 %), *Bacilli* (1.95 %), *Bacteroidia* (1.35 %), *Clostridia* (0.78 %), and *Flavobacteria* (0.58 %).

The distribution of the class into different order further revealed that they were shared into 12 top order namely: *Burkholderiales* (47.13 %), *Enterobacteriales* (25.65 %), *Pseudomonadales* (19.65 %), *Xanthomonadales* (1.50 %), *Lactobacillales* (1.4 %), *Rhizobiales* (1.38 %), *Flavobacteriales* (1.04 %), *Caulobacteriales* (0.81 %), *Clostridiales* (0.54 %), and *Bacteroidales* (0.45 %). The distribution of order into different families further revealed that they were shared into 13 top families as follows: *Alcaligenaceae* (43.67 %), *Enterobacteriaceae* (25.65 %), *Moraxellaceae* (19.65 %), *Comamonadaceae* (3.46 %), *Xanthomonadaceae* (1.5 %), *Enterococcaceae* (1.4 %), *Brucellaceae* (1.29 %), *Weeksellaceae* (0.85 %), *Caulobacteraceae* (0.81 %), *Morganellaceae* (0.48 %), *Sphingobacteriaceae* (0.43 %), *Microbacteriaceae* (0.28 %), *Flavobacteriaceae* (0.19 %), *Bacteroidaceae* (0.26 %), and *Porphyromonadaceae* (0.1 %). Further distribution of the family into genus revealed that they were affiliated to top 18 bacterial genera as shown in figure 7. The top 18 genera OTUs in the consortium were revealed in the order which showed that *Alcaligenes* had the highest relative abundance of 48.28 % followed by *Acinetobacter* (13.97 %), *Morganella* (5.38 %) and others while the lowest was *Elizabethkingia* which had 0.215 % relative abundance.

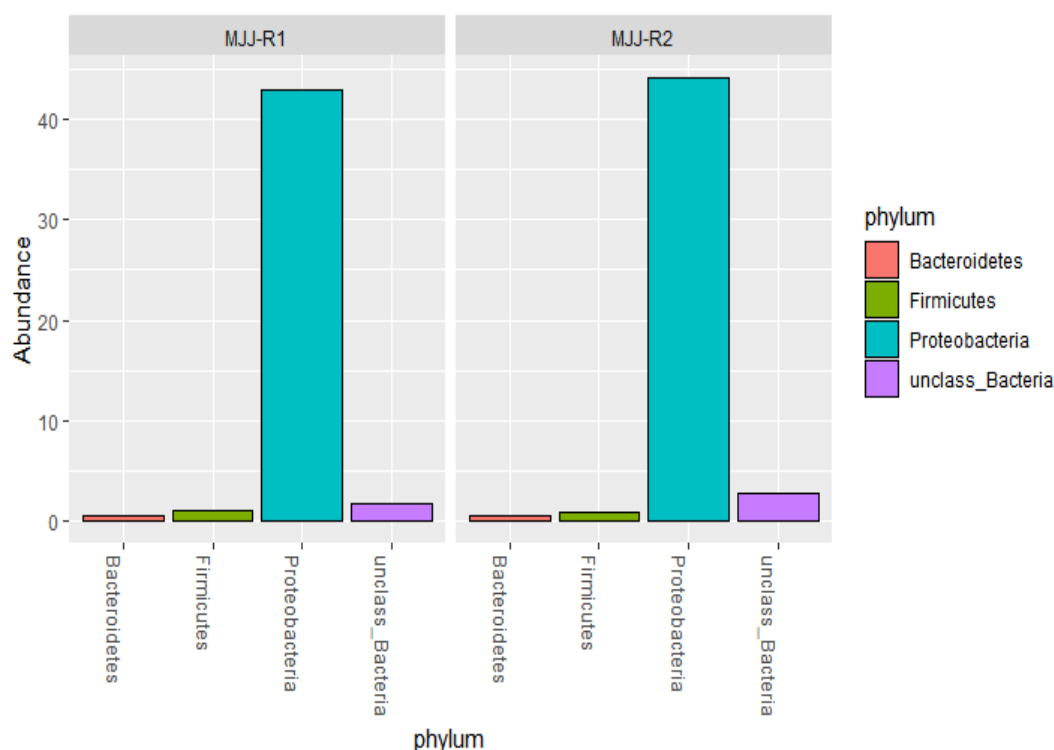


Figure 6: Relative abundance of the phyla observed in the bacterial consortium

The top 5 dominant phyla that make up the consortium were analyzed for their distribution from phylum to genus level using a heat map. The heat map showed that the bacterial diversity as it is affiliated to the different bacterial genera. They are presented into distinct clusters based on their relative taxonomic distribution of normalized abundance in 6

microcosm metagenome. The top 12 genera OTUs that make up the consortium were revealed in the order which showed that the highest relative abundance from phylum two genus level was *>Bacteria- Proteobacteria - Gammaproteobacteria - Pseudomonadales - Moraxellaceae - Acinetobacter* while the least relative abundant was *>Bacteria - Verrucomicrobia -*

Verrucomicrobiae - *Verrucomicrobiales* - *Verrucomicrobiaceae* - *Akkermansia* as shown in figure 8.

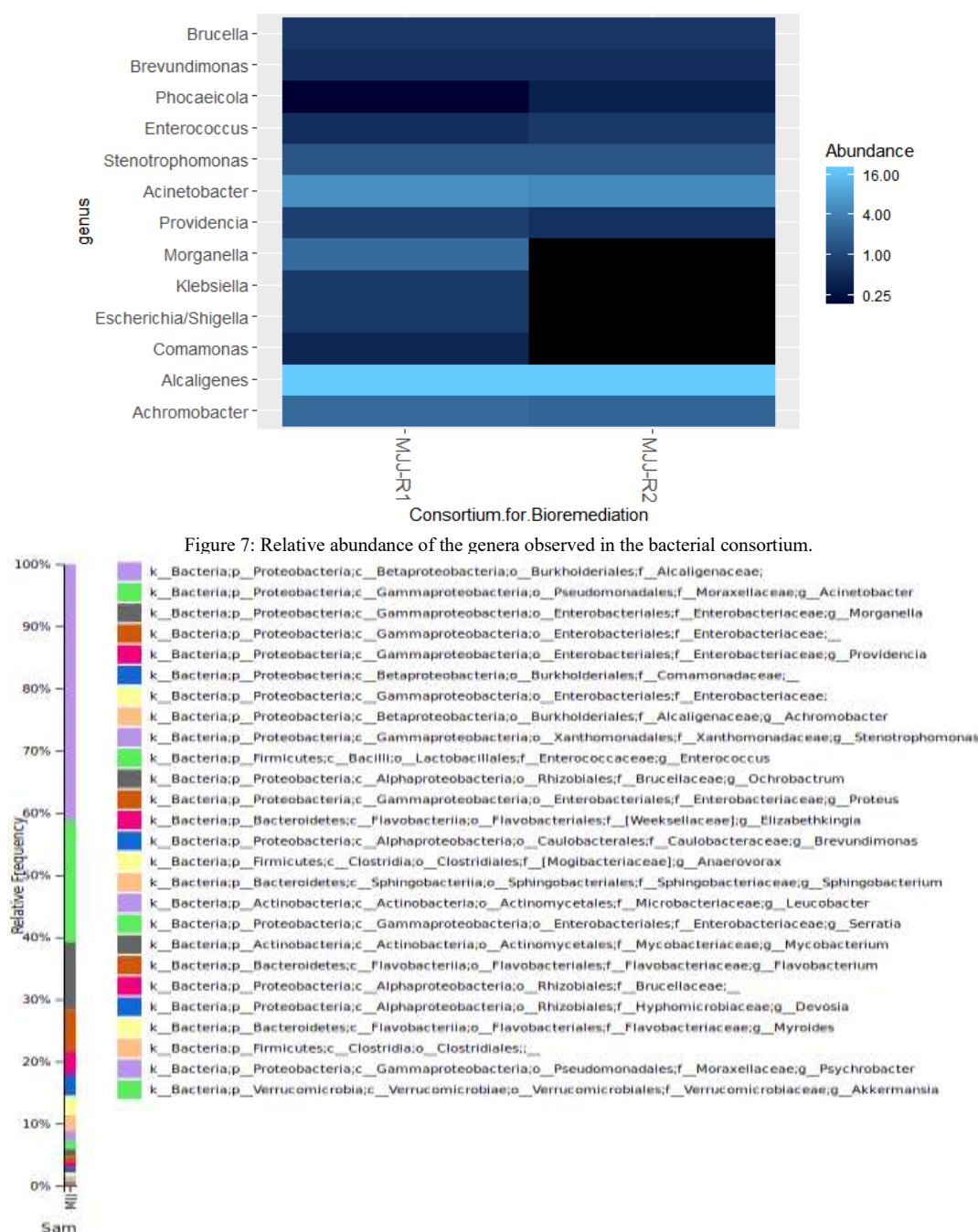


Figure 8: Relative frequency of the taxa present in the bacterial consortium

IV. DISCUSSION

Bioremediation have proved to be a reliable, safe and cost-effective approach for the management of crude oil-pollution. However, its success is highly dependent on the indigenous microbial population. The ability of these indigenous microbial endophytic populations to possess degradative potential can be influenced by nutrient availability and other *in situ* and *ex situ* physicochemical and environmental conditions (Maduwuba and Ubani, 2024; Chikere *et al.*, 2019).

Studies have shown that most of these endophytes in exotic sites are non-culturable though viable and as such can be lost during culture dependent process. Little information is available on the analysis of endophytic bacterial communities present in abandoned crude oil polluted sediment using the 16S amplicon sequencing (dos Santos *et al.*, 2011; Abed *et al.*, 2014; Chikere *et al.*, 2019).

It was observed that the pH of the sediment samples were low (slightly acidic). During the bioremediation treatment experiment, pH ranged from 6.28 ± 0.16 to 7.1 ± 0.098 . This pH range corresponds to the findings of Chikere *et al.* (2009)

and Ukaegbu-obi and Omeh (2014) where it was revealed that most polluted sites exhibit slightly acidic to neutral pH. This could be due to the time interval between the oil spill incident and the sampling time. The acidity also implies that the environment still contained the acidic elements of the crude oil. The neutral to slightly alkaline pH could also have resulted from the degradation of hydrocarbon components present in the sediment samples suggesting that the reduction in TPH gives rise to a neutral to alkaline environment.

The TPH concentrations of the samples were high and exceed DPR intervention values for micro-pollutants. The high TPH concentration in the samples could be due to the degree and duration of pollution. There was reduction in TPH concentration of the different treatment setups within the 42 days period. Sample A (negative control) experienced the lowest TPH loss of 4.51 %, 6.04 % and 8.40 % at 14, 28, and 42 respectively because it was unamended while sample C recorded the highest TPH loss of 84.13 % at day 14, 89.17 % at day 28 and 93.17 % at day 42 because it was biostimulated. This is an indication of the presence of metabolically active endophytic bacterial community which has the ability to metabolize hydrocarbons when stimulated through the addition of nutrient supplements (Orji *et al.*, 2012; and Ezekoye *et al.*, 2018).

The presence of bacterial activity in the samples was monitored by enumerating the total culturable heterotrophic bacterial counts (TCHBC) and total culturable hydrocarbon utilizing bacterial counts (TCHUBC). This revealed that apart from the hydrocarbon utilizers, other endophytic bacteria were able to thrive and survive in the polluted environment due to prolonged exposure and adaptation to their environment. The highest TCHBC was recorded in sample D on day 28 while for TCHUB, the highest endophytic hydrocarbon utilizing population was recorded in sample C on day 28.

The result of Peng *et al.* (2015) on the bacterial community diversity of oil-polluted lands showed that *Acidobacteria*, *Acintobacteria*, *Proteobacteria*, *Bacteroidetes*, *Chloroflexi* and *Planctomycetes* were the major bacterial groups present. However as shown in this study, the top 4 phyla were *Proteobacteria*, *Bacteroidetes*, *Firmicutes* and *Unclass_Bacteria* among which *Proteobacteria* were the most abundant and this interaction have been recorded in other impacted environments (Chikere *et al.*, 2019; Galazka *et al.*, 2018). Also, a study done by Gkorezis *et al.* (2016) reported the phylum *Proteobacteria* as a distinct bacteria group which includes hydrocarbon utilizers and plant growth promoting bacterial (PGPB) species due to the possession of degradative genes and enzymes that helps the organism to break down complex substances present in petroleum compounds during metabolism (Bruto *et al.*, 2014). The high abundance of the phylum *Firmicutes* found in the samples could be attributed mainly to the pH value of the samples. The acidic pH of the sites created an enabling environment for acid-loving bacteria to thrive. The presence of *Unclass_Bacteria* in this environment could suggest that they may be outstanding novel bacterial species that needs further studies on their classification and capabilities in bioremediation technology.

The presence of the order *Pseudomonadales* and *Acidobacteriales* in significantly differential abundance can be

linked to the slightly acidic pH present in the samples and in most hydrocarbon-polluted sites in Ogoniland and Niger Delta at large as reported by the United Nations Environmental Programme (UNEP). The *Pseudomadales* have been shown to exhibit more versatile catabolic potentials and are good PCB (Polychlorinated biphenyls) degraders especially in natural environments. The high abundance of the genera *Alcaligenes* and *Acinetobacter* revealed that they are the major players in the degradation of hydrocarbons during the treatment period. The major reason for the different treatments was to compare the effectiveness of the indigenous bacterial endophytes (bioaugmentation) with different nutrient amendment (biostimulation).

Overall, the results of this study has revealed the ability of endophytic bacterial communities in crude oil polluted mangrove ecosystem to degrade and restore crude oil-contaminated environment and the need to harness such organisms in the restoration of polluted systems. However, more study is needed to investigate the specific functional and catabolic genes possessed by each member of this bacterial community so that they can be effectively and efficiently employed in the restoration and recovery of polluted environments.

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