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BIOREMEDIATION EFFICIENCY OF *Exiguobacterium Profundum* AND *Bacillus Thuringiensis* IN THE REMEDIATION OF BITUMEN

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ABSTRACT

Industrial activities are major contributors to environmental pollution, particularly in soil and aquatic ecosystems. As petroleum remains a primary energy source, its by-products, such as bitumen, are prevalent environmental contaminants. This study investigates the bioremediation potential of *Exiguobacterium profundum* and *Bacillus thuringiensis* in degrading bitumen. These bacteria were isolated from bitumen-contaminated soil in Ondo State, Nigeria, and identified through standard biochemical and molecular techniques. After 30 days of incubation, both strains exhibited significant bitumen-degrading capabilities. GC-MS analysis revealed that *E. profundum* (PP278055) transformed n-alkanes (C3–C20) into fatty acids including hexadecanoic acid, oleic, octadecanoic acid, pentadecanoic acid, octanoic acids and octadecenal compounds. Similarly, *B. thuringiensis* (PP278059) degraded n-alkanes (C5–C19) into acetic, hexadecanoic acid, oleic acids, and octadecenal compounds. These transformations indicate effective hydrocarbon biodegradation. Quantitative analysis showed that *E. profundum* achieved a higher bitumen weight loss (36.2%) compared to *B. thuringiensis* (31.6%). These findings suggest that both bacterial strains, particularly *E. profundum*, hold promise for the bioremediation of bitumen-contaminated environments.

Keywords: Bitumen degradation, Hydrocarbon pollution, *Exiguobacterium profundum*, *Bacillus thuringiensis*, GC–MS analysis, n-alkanes, Bitumen-contaminated soil, Nigeria

INTRODUCTION

The rise of urbanization and industrialization, has left the environment exposed to numerous pollutants which are toxic to living things. Pollutants arising from different industrial processes are major sources of pollution to the soil and aquatic environment. Different types and quantities of heavy metals are released during the industrial production process and as effluents after further industrial production (Methneni *et al.*, 2021). Petroleum continues to serve as the principle source of energy, and had made it a contaminant in both prevalence and quantity in the environment. It is a complex mixture of hydrocarbons formed from the geologic transformation and decomposition of plants and animals that lived hundreds of millions of years ago (Gbolahan-Ayoade *et. al*., 2014). Generally, petroleum encompasses the liquid (crude oil), gaseous (natural gas), and viscous or solid (bitumen asphalt) forms of hydrocarbons that occur in the Earth, however, the meaning is often restricted to the liquid oil form.

Nigeria is blessed with vast deposits of natural bitumen (Adegoke, 2000) with a proven reserve of 42.47 billion metric tonnes, the second largest in the world, covering about 120×4.3 km (Oboh *et. al*., 2026). Crude oil is one of the most important natural resources in our modern life but a major fraction of economically available oil exists as biodegraded heavy oil or bitumen (Head *et. al.*, 2003). Bitumen are solid or semisolid hydrocarbons which are sticky, black and highly viscous (Strausz *et. al*., 2010). It exhibits thermoplastic behaviors softening when heated, becoming mobile liquids on further heating and returning to their original state on cooling. They are naturally occurring substances that are considered to be complex mixtures of high-molecular-weight hydrocarbons with relatively low hydrogen to carbon ratio (Yoon *et. al*., 2009) and non-hydrocarbons which can be separated into fractions consisting of oily material, resins, asphaltenes, and carbenes. These fractions merge into one another and their atomic C/H ratio increases with each succeeding member, except for the carbenes which differ mainly in having more oxygen than the asphaltenes. Bitumen is classified into following groups: Mineral waxes, Asphalts, Asphaltites and Oil-shale bitumen. Bitumen mixed with mineral matter is defined as asphalt. Three types of hydrocarbons are present in bitumen: paraffinic, naphthenic, and aromatic hydrocarbons. Non-hydrocarbons in bitumen have heterocyclic atoms consisting of sulphur, nitrogen, iron and vanadium (Spirov *et. al*., 2013; Oguntimehin and Ipinmoroti, 2007).

Microbial processes can minimize the generation of harmful byproducts, thereby reducing the overall environmental impact (Singh et al., 2022). In addition, microorganisms can adapt and evolve to survive in contaminated environments, providing long-term remediation solutions that continually improve over time (Giovanella et al., 2020). The mechanism of bioremediation in cludes reduction, detoxification, degradation, mineralization, or transformation of toxic pollutants to less toxic/hazardous forms (Dukda and Adriano, 1997; Yakasai et al., 2019). During the degradation process, n-alkanes, monocyclic alkanes, and alkyl benzenes disappear first, which leads to a lower oil quality and finally to natural bitumen mostly consisting of saturated and aromatic hydrocarbons, resins, and asphaltene. {Kayukova et. al., (2016); Mbadinga et. al., (2011); Larter et. al., (2003)}.

The genus *Bacillus* Are Gram-positive, spore-forming, rod-shaped, and aerobic or facultative anaerobes, most commonly found in soil but can also be isolated from other sources, e.g., water. The unique trait of *Bacillus* spp. is the ability of spore-forming under extreme conditions. Due to their specific structure, the spores are able to resist significant environmental stresses, including high temperature, drought, humidity, and radiation. This characteristic gives them an advantage over other bacteria and makes them eagerly used commercially in various fields of industry and agriculture {Pham et. al., 2002; Elshaghabee et. al., 2017}. The genus *Exiguobacterium* is renowned for its widespread distribution and remarkable adaptability, enabling it to thrive in diverse marine and terrestrial habitats. They are known for their thermophilic, psychrophilic, basophilic, halophilic, and xerotolerant characteristics, which form the basis for their

survival in extreme environmental conditions, including high salinity, low temperature, high radiation, and desiccation (Gutierrez-Preciado et al., 2017) showing their significant potential for the removal of environmental pollutants.

The vandalization, leakage and/or accidental spillage of these petroleum hydrocarbon are toxic to aquatic and terrestrial habitats and their inhabitants. Therefore, there is need for the remediation of these pollutants using physical, chemical, or biological methods. The physical and chemical methods have been used for years but they come with their drawbacks which include the need for an expert and special equipment for the chemical bioremediation procedure while the physical bioremediation procedure is expensive (Mahmood et al., 2021). This has called for the need for a better alternative which is the biological remediation (Bioremediation). Bioremediation is a most efficient, eco-friendly and cost effective technology for the transformation of contaminants (Sonune, 2021) when compared to other technologies. These processes rely on the natural ability of microorganisms to carry out the mineralization of organic chemicals. Among several clean-up techniques available to remove petroleum hydrocarbons from the soil and groundwater, bioremediation processes are gaining ground due to their simplicity, higher efficiency and cost-effectiveness (Alexander, 1994). The aim of this study is to investigate the remediative potential of indigenous bacteria (*Exiguobacterium profundum* and *Bacillus thuringiensis*) in the bioremediation of bitumen polluted soil

MATERIALS AND METHODS

Description of Sample Location

Agbabu is a town situated in Odigbo, Local Government Area of Ondo State, Nigeria, known primarily for its significant bitumen deposits. It is located in the southern part of the state; it is a key area for the country's bitumen industry.

Collection of Sediment and Water Samples

Sediment and water samples were collected from various locations within Agbabu village, located in Odigbo Local Government Area of Ondo State, Nigeria. The sampling area lies within the geographical coordinates of latitude 6°35'148'N to 6°37'248" and longitude 4°49'766"E to 4°49'830"E (Figure 1). All samples were collected using sterile equipment and following standard environmental sampling protocols. Sampling was conducted to account for potential variations in microbial populations and petrochemical contamination levels**.**

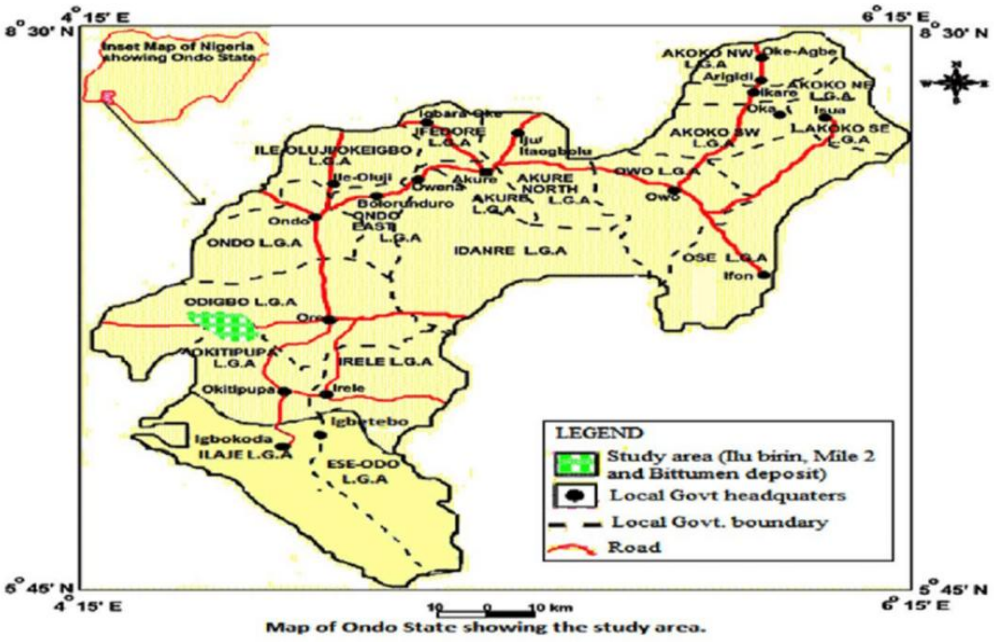

Figure 1: Sampling Site: Agbabu, Odigbo L.G.A., Ondo State (Olowomofe *et al*., 2019)

Water Samples

Surface water samples were collected from polluted areas using sterile glass wares. Three sampling stations were established, each 200 meters apart. At each station, water samples were collected according to standard methods for the examination of water and wastewater.

Sediment Samples

Sediment samples were collected from the same three sampling stations using a sterile auger. Samples were taken from a depth of 2-3 cm below the surface to ensure collection of the active microbial layer. As a control, additional samples were collected from a randomly selected well approximately 120 meters away from the contaminated sites (Oboh *et al*., 2006). All samples were collected in triplicate, stored in sterile containers, and transported to the laboratory in coolers with ice packs to maintain a temperature of 4°C. Samples were processed within 24 hours of collection to ensure the integrity of the microbial communities.

Isolation and Identification of *Bacillus thuringiensis* and *Exiguobacterium profundum*

Culture Media Preparation

For the isolation of *Bacillus thuringiensis and Exiguobacterium profundum*, Mineral Salt Medium (MSM) was used for culturing and the MSM composition was as follows: K_2HPO_4 1.73 g, KH_2PO_4 0.68g, $MgSO_4{\cdot}7H_2O$ 0.1g, $FeSO_4{\cdot}7H_2O$ 0.03g, NH_4NO_3 1.0 g, $CaCl_2{\cdot}2H_2O$ 0.02 g, and NaCl 4.0 g, with a final pH adjusted to 7.0.

Isolation Techniques

Aseptic conditions were maintained throughout experimental procedures; the laboratory bench was sterilized using cotton wool soaked in ethanol to maintain aseptic conditions. The sediment samples were carefully weighed, and 1.0 g of each sediment sample was subjected to serial dilution. Similarly, 1.0 ml of each water sample underwent a ten-fold serial dilution. For the serial dilution process, 9 ml of sterile distilled water was dispensed into each of 10 test tubes. Subsequently, 1.0 g of soil or 1 ml of water was introduced into the first test tube, which contained 9 ml of sterile distilled water, and the contents were thoroughly mixed. From this first test tube, 1 ml of the mixture was transferred into the second test tube, mixed and this process was repeated, continuing the ten-fold serial dilution.

The pour plate method was employed to evaluate the bacterial content in the dilutions. Aliquots from four dilutions (10^{-4}, 10^{-5}, 10^{-6}) were plated. For each dilution, 1 ml of the aliquot was transferred into appropriately labeled sterile Petri dishes, followed by the addition of molten sterilized Mineral Salt Medium (MSM). The MSM composition was as follows: K_2HPO_4 1.73 g, KH_2PO_4 0.68g, $MgSO_4 \cdot 7H_2O$ 0.1g, $FeSO_4 \cdot 7H_2O$ 0.03g, NH_4NO_3 1.0 g, $CaCl_2 \cdot 2H_2O$ 0.02 g, and NaCl 4.0 g, with a final pH adjusted to 7.0. The plates were gently rocked to ensure uniform distribution of the medium and were then allowed to solidify.

After solidification, the plates were incubated aerobically and anaerobically at 37°C for 24 hours. The bacterial colonies on each plate were counted and recorded.

Molecular Identification of Bacteria Isolates IID and IIF

Genomic DNA Isolation

Isolate IID AND IIF were molecularly characterized by analyzing the 16S conserved region of the bacteria. The genomic DNA of the overnight grown culture of IID and IIF were isolated using Quick Fungal/Bacteria DNA miniprep kit (Zymo Research, USA) as described by Ogundolie (2022).

PCR Amplification, Sequencing and Data Analysis

To amplify the 16S conserved region of the genomic DNA (gDNA) of isolate IID and IIF, a 25 µL reaction volume that contains the PCR Master mix, gDNA as template, nuclease-free water and universal primers (27F: 5'-AGAGTTTGATCCTGGCTCAG-3') and 1392R: 5'-GGTTACCTTGTTACGACTT-3') was prepared. The amplification was achieved using a Veriti thermal cycler (Thermo Fishers, USA). using under the following reaction conditions; initial denaturation (94℃; 30 seconds), 32 cycles of denaturation (94℃; 30 seconds), annealing (45℃; 55 Seconds), initial extension (68℃; 60 seconds), final extension (68℃; 7 minutes) followed by holding (4-8℃). Amplicons were loaded on 1% Agarose gel electrophoresis and purified before the PCR product was subjected to Sanger sequencing. Nucleotide sequences obtained were analyzed using various bioinformatics tools such as ChromasPro DNA Sequencing Software, BioEdit Sequence Alignment Editor, and Basic Local Alignment Search Tools (BLASTn) respectively. Evolutionary relationship of the isolate was analysed using MEGAX (Molecular Evolutionary Genetics AnalysisX) (Ogundolie, 2024).

Bitumen Preparation for Bioremediation

Fifty grams (50 grams) of bitumen was carefully weighed and transferred to a clean glass container. An appropriate volume of Dichloromethane (DCM) was then added to the bitumen in a weight-to-volume ratio of 1:10, ensuring sufficient solvent to completely dissolve the bitumen.The mixture was stirred continuously, for 25-35 minutes, using a glass rod or mechanical stirrer until the bitumen was fully dissolved in the DCM, forming a uniform solution.

Experimental Setup for Bioremediation

The conical flasks were labeled for different bitumen treatments. 50 g of the prepared soil sample was added to each flask. 5mL of bitumen was added to the respective flasks and mixed thoroughly with soil. 100 ml of sterile nutrient broth was added to each flask to create slurry.

For treatments, 5 ml of the standardized bacterial suspension was added to the respective flasks. For control flasks, only the nutrient broth was used. The flasks were covered tightly with sterile caps to avoid contamination. They were then placed in a water shaker set at 150 rpm and 30-35°C. The samples were incubated for 30 days.

Quantification of Biodegradation Efficiency

The extent of the utilization of hydrocarbon in the bitumen was estimated gravimetrically on the 30th day of incubation. This was achieved by harvesting the residual bitumen from both the control and experimental set-ups, using modified method of Olabemiwo et al., (2011a). 30mL of dichloromethane (DCM) was added to the culture and shaken vigorously for 5 min to extract the residual bitumen.

The biodegradation efficiency was calculated using the following formula: Biodegradation efficiency (%) = [(Initial oil content - Residual oil content) / Initial oil content] × 100

GC-MS Analysis

For the GC-MS analysis of soil and water samples, the process began with sample collection, where 50–100 g of soil and 100–200 ml of water were collected, labeled, and stored at 4°C. Soil samples were extracted using organic solvents through sonication and centrifugation, while water samples were extracted using a separatory funnel. The resulting organic layers were concentrated, and if necessary, derivatization was performed to modify specific analytes. The 25 GC-MS was then set up with an appropriate column and mass spectrometer settings, followed by the injection of 1–2 µl of the extract for analysis. The chromatograms and mass spectra were used to identify and quantify compounds by comparing results with reference libraries. Quality control was maintained through blanks, standards, and replicates to ensure accuracy. Safety measures, including the use of PPE and proper solvent disposal, were followed throughout the procedure (EPA, 2014).

Data Analysis

The rate of hydrocarbon degradation between different treatments was compared. The efficiency of *B. thuringiensis* and *E. profundum* in remediation was evaluated compared to control samples.

RESULTS AND DISCUSSION

Table 1: Biochemical Identification of Isolates IID and IIF

Biochemical Characteristics	IID (*Exiguobacterium profundum*)	IIF(*Bacillus thuringiensis*)
Catalase	+	+
Citrate	+	+
Gram Staining	+	+
H_2S	-	+
Indole	-	-
Motility	-	+
Oxidase	-	+
Shape	Rod	Rod
Spore	-	+
VP (VogesProskauer)	-	+
MR (Methyl Red)	+	-
Starch	-	+
Sugar Fermentation of		
Glucose	+	+
Lactose	-	-
Sucrose	-	+
Colony form	Irregular	Circular
Colony color	Cream	White
Elevation	Raised	Flat
Margins	Smooth	Irregular

Keys: + = positive, - = negative

The biochemical characterization results (Table 1) showed that *Bacillus thuringiensis* is a Gram-positive, catalase-positive, oxidase-positive rod-shaped bacterium capable of forming spores. It demonstrated positive for citrate utilization, motility, starch fermentation abilities, H_2S production, and the Voges-Proskauer test, while testing negative for the Methyl Red test and indole production. Sugar fermentation revealed that the isolate could ferment glucose and sucrose, but not lactose. In addition, biochemical results showed that *Exiguobacterium profundum* is also a Gram-positive, catalase-positive, oxidase-negative rod-shaped bacterium incapable of forming spores. It demonstrated positive for citrate utilization and the Methyl Red test, while testing negative for the Voges Proskauer test, H_2S production, motility, starch formation abilitiy. Sugar fermentation revealed that the isolate could ferment glucose but not sucrose and lactose. The colonial morphology of these isolates showed that *Bacillus thuringiensis* exhibited a circular, flat, colony with white appearance and irregular margins while *Exiguobacterium profundum* exhibited an irregular, cream coloured appearance with raised elevation and smooth margins. These are consistent with the typical profile of *Bacillus thuringiensis* and *Exiguobacterium profundum*

Table 2: Molecular Identity of Isolates IID and IIF

Isolate Code	Molecular Identity	Accession number
IID	*Exiguobacterium profundum*	PP278055
IIF	*Bacillus thuringiensis*	PP278059

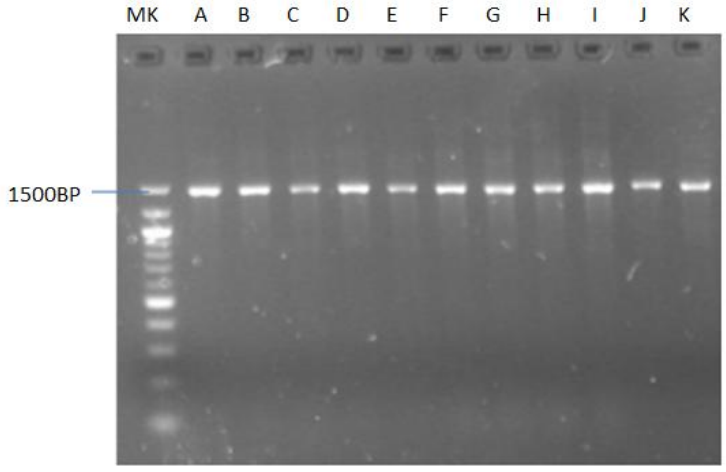

Fig. 1: Agarose Gel Electrophoresis Analysis of PCR Reaction for DNA Extracted

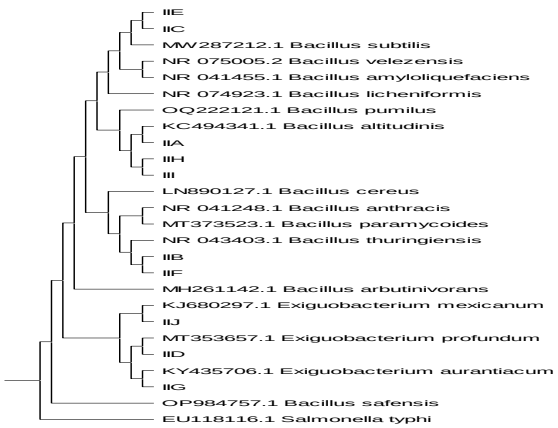

Fig. 2: Phylogenetic Tree of Isolates IID and IIF

AACCTACCGGAGGCACCCGTGGGGAATCTTCCCCAATGGACGAAAGTCTGATGGAGCAACCCCGCGTGAACG
ATGAAGGCTTTCGGGTCGTAAAGTTCTGTTGTAAGGGAAGAACAAGTGCCGCAGGCAATGGCGGCACCTTGA
CGGTACCTTGCGAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGT
CCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGCCTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGG
GAGGGCCATTGGAAACTGGGAGGCTTGAGTATAGGAGAGAAGAGTGGAATTCCACGTGTAGCGGTGAAATGC
GTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTTTGGCCTATAACTGACGCTGAGGCGCGAAAGCG
TGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAGGTGTTGGAGGGTTTC
CGCCCTTCAGTGCTGAAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGGCTGAAACTCAAAG
GAATTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCAAAGCAACGCGAAGAACCTTACCAAC
TCTTGACATCCCCCTGACCGGTACAGAGATGTACCTTCCCCTTCGGGGGCAGGGGTGACAGGTGGTGCATGGT
TGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCTTAGTTGCCAGCA
TTTGGTTGGGCACTCTAGGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATG
CCCCTTATGAGTTGGGCTACACACGTGCTACAATGGACGGTACAAAGGGCAGCGAAGCCGCGAGGTGGAGCC
AATCCCAGAAAGCCGTTCTCAGTTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGTCGGAATCGCTAGTAAT
CGCAGGTCAGCATACTGCGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCACGAGAGTTTGC
AACACCCGAAGTCGGTGAGGTAACCGTAAG
DNA Sequence of *Exiguobacterium profundum*

GAAGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAACCG
GGGCTAATACCGGATAACATTTTGAACTGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGAT
GGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAG
GGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCA
ATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGG
GAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTG
CCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGT
TTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGA
AGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCG
ACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCC
ACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCACT
CCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCAT
GTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGAAAACCCTAGAGATAGGGCT
TCTCCTTCGGGAGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTT
DNA Sequence of *Bacillus thuringiensis*

Bioremediation Activity

For confirmation of hydrocarbon degrading activity in MSM GC-MS analysis of control (bitumen without bacteria) was done which showed it was a mixture of different hydrocarbons and further it was compared with GCMS results of bitumen extracts from inoculated medium. The evaluation of the bitumen biodegradation by *Bacillus thuringiensis* and *Exiguobacterium profundum* was carried out during the process of 30 days using gas chromatography with mass spectrometry GC/MS. After 30 days of incubation, the undegraded bitumen residue was extracted twice with equal volumes of dichloromethane (DCM). The results showed appearance of new compounds through bio-degradation with less molecular weight and less complex such as carboxylic acids and alcohols.

Table 3: Major Molecular Fragmentation of Bitumen Sample Before Degradation

Retention Time (R.Time)	Formula	Compound detected
7.008	$C_{10}H_{22}$	Decane
8.57	$C_9H_{16}N_2$	Pyrimidine
8.823	$C_{10}H_{22}$	Decane
10.064	$C_{11}H_{24}$	Octane
10.657	$C_{13}H_{28}$	Tridecane
12.077	$C_{16}H_{34}$	Hexadecane
12.278	C18H34	9-Octadecyne
12.509	$C_{13}H_{28}$	Tridecane
13.5	$C_{12}H_{26}$	3,7-dimethyl decane
14.102	$C_{16}H_{34}$	n-Cetane
15.496	H_2O	Water
16.097	$C_{18}H_{38}$	Pentadecane
16.745	$C_{19}H_{19}NO_3$	Propanamide
16.79	$C_{15}H_{32}$	Dodecane
17.89	$C_{16}H_{34}$	Hexadecane
17.974	$C_{17}H_{36}$	Heptadecane
18.955	$C_3H_6N_6$	1,3,5-Triazine-2,4,6-triamine
20.002	$C_{11}H_{24}$	Undecane
21.239	$C_{20}H_{42}$	Eicosane
22.749	$C_{16}H_{34}$	Hexadecane
24.007	CH_3CLO_2S	Methanesulfonyl chloride
25.005	$C_{13}H_{28}$	4-Ethylundecane
25.843	CH_2BrNO_2	Bromonitromethane
26.578	$C_{15}H_{32}$	2,6,11-Trimethyldodecane

Table 4: Hydrocarbon Compounds Detected from Bitumen-Bioremediated Samples Using *Exiguobacterium Profundum*

Compound detected	Formula	Retention Time (R.Time)	Area (%)
Decanoic acid,	$C_{11}H_{22}O_2$	15.713	1.41
Hexadecanoic acid	$C_{18}H_{36}O_2$	16.357	1.98
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	16.44	9.05
11-Octadecenoic acid	$C_{19}H_{36}O_2$	17.427	6.45
Octanoic acid	$C_9H_{18}O_2$	17.628	1.23
9- Octadecanoic acid	$C_{20}H_{38}O_2$	18.013	14.79
Oleic Acid	$C_{18}H_{34}O_2$	18.17	43.81
Pentadecanoic acid	$C_{15}H_{30}O_2$	18.304	11.54
6-Octadecenoic acid,	$C_{18}H_{34}O_2$	19.665	6.6
9-Octadecenal	$C_{18}H_{34}O$	20.727	3.15

Table 5: Hydrocarbon Compounds Detected from Bitumen-Bioremediated Samples Using *Bacillus Thuringiensis*

Compound detected	Formula	Retention Time (R.Time)
n-Hexadecanoic	$C_{16}H_{32}O_2$	20.866
Oleic Acid	$C_{18}H_{34}O_2$	23.667
9-Octadecenal	$C_{18}H_{34}O$	28.979

Table 3 showed that bitumen sample contained majorly n-alkanes, branched alkanes, and small concentrations of aromatic polycyclic compounds. *Exiguobacterium profundum* and *Bacillus thuringiensis* were capable of utilizing a wide range of hydrocarbons, with a preference for alkanes with intermediate carbon chain lengths as shown in (Table 4 and 5). These isolates were found actively able to degrade total mixture of hydrocarbons present in the bitumen contaminated soil samples. The result was confirmed by almost total disappearance of the corresponding peak of each compound and the transformation of the n-alkanes {Kayukova *et. al*., (2016); Mbadinga *et. al*., (2011); Larter *et. al*., (2003)}. It is expected that the hydrocarbon assimilating capabilities in the medium is due to adaptation of isolate due to previous exposure to hydrocarbons. It may indicate the ability of the emulsification of hydrocarbons, which is a major factor for hydrocarbon uptake and assimilation.

Bioremediation Efficiency

Table 6: Percentage Weight Loss of Bitumen After Bioremediation

Isolates	% weight loss
IID (*Exiguobacterium profundum*)	36.2
IIF (*Bacillus thuringiensis*)	31.6

The above gravimetric analysis shows significant weight loss observed in the treated samples compared to the control which suggests their potential for application in bitumen polluted sites.

CONCLUSION

This study focused on bacterial isolates, *Exiguobacterium profundum* and *Bacillus thuringiensis*, isolated from bitumen contaminated site from Agbabu, Odigbo LGA, Ondo state, Nigeria. In this study, the isolated bacteria have been shown to degrade a wide range of hydrocarbons and completely metabolize n-alkanes. From the data presented in this study, it can be concluded that *Exiguobacterium profundum* and *Bacillus thuringiensis* can be considered as good prospects for their application in bioremediation of bitumen contaminated sites.

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