Characterization of *Exiguobacterium Profundum* Isolated From Petrochemical Waste (Bitumen) For Bioremediation and Bioelectricity Production Using Microbial Fuel Cell Technology

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ABSTRACT

The persistent contamination of soil and water by bitumen and other petrochemical wastes presents serious environmental and public health challenges, particularly in regions with natural hydrocarbon deposits such as Agbabu, Ondo State, Nigeria. This study aimed to isolate, characterize, and evaluate the bioremediation and bioelectricity generation potential of *Exiguobacterium profundum* recovered from bitumen-contaminated sediments. Standard microbiological techniques were employed for isolation, followed by biochemical characterization and molecular identification using 16S rRNA gene sequencing, which confirmed the presence of the isolate *Exiguobacterium profundum* (Accession No. PP278055). Biodegradation efficiency was assessed over a 30-day incubation period using gravimetric analysis and Gas Chromatography–Mass Spectrometry (GC-MS). The isolate achieved a 36.2% reduction in bitumen weight, with GC-MS results indicating transformation of complex hydrocarbons such as decane, hexadecane, and eicosane into simpler compounds including fatty acids (oleic acid, hexadecanoic acid, and octadecanoic acid), confirming active metabolic degradation pathways. Furthermore, the organism was evaluated in a constructed dual-chamber Microbial Fuel Cell (MFC) system using potassium permanganate as a mediator. The system generated an initial peak voltage of 1.11 V and current of 0.31 A, although outputs gradually declined over 14 days, likely due to substrate depletion and reduced microbial activity. The findings demonstrate that *E. profundum* possesses significant hydrocarbon-degrading and electrogenic capabilities, highlighting its dual applicability in sustainable bioremediation and bioelectricity production. This study establishes the potential of indigenous microbial resources as cost-effective and environmentally friendly tools for integrated petrochemical waste management and renewable energy recovery.

Keywords: Bioremediation, Bioelectricity, *Exiguobacterium profundum,* Microbial Fuel Cell

INTRODUCTION

The escalating global demand for energy has inherently resulted in substantial environmental contamination stemming from unconventional hydrocarbon resources, notably bitumen. Found in extensive deposits, such as those in Nigeria's Ondo State, the extraction, processing, and natural seepage of this dense, viscous petroleum contribute to pervasive soil and water pollution (Akinbebije *et al*., 2024). This contamination is characterized by a complex matrix of highly recalcitrant polycyclic aromatic hydrocarbons (PAHs), alkanes, and heterocyclic compounds, which pose acute and chronic toxicity risks to both ecosystems and human health (Haritash and Kaushik, 2008).

Conventional remediation technologies, including thermal and chemical treatments, are frequently resource-intensive, economically unfeasible, and can potentially introduce secondary contaminants into the environment (Vidali, 2001). Consequently, the imperative to develop sustainable, cost-effective, and ecologically sound alternatives is paramount. Bioremediation, which harnesses the metabolic capacity of microorganisms to

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degrade and detoxify pollutants, represents the most promising solution (Chikere *et al*., 2009). The efficacy of this approach relies fundamentally on the identification and characterization of robust microbial strains capable of proliferating within the harsh, nutrient-limited, and toxic conditions typical of a petrochemical waste environment. The sampling location, Agbabu in Ondo State, Nigeria, is a site of chronic natural bitumen contamination, which serves as a potent natural selection ground for specialized, resilient microbial communities, often classified as extremotolerant organisms (Zhou *et al*., 2023). Such environments favor microorganisms possessing sophisticated enzymatic machinery necessary to initiate the oxidative degradation of complex hydrocarbon structures.

This study focuses on the isolation and comprehensive characterization of a potent hydrocarbon-degrading bacterium, identified as *Exiguobacterium profundum*, recovered directly from this bitumen-rich sediment. The genus *Exiguobacterium* is renowned for its remarkable metabolic versatility, having been isolated from diverse extreme niches, including psychrophilic and halo-tolerant environments (Vishnivetskaya *et al*., 2011). *Exiguobacterium profundum*, a Gram-positive bacterium known for its versatility in extreme environments, has attracted attention for its potential in degrading complex hydrocarbons. The presence of *E. profundum* in this context suggests an exceptional capacity to adapt to the high-hydrocarbon, potentially oligotrophic, and semi-anoxic conditions characteristic of bituminous waste sites. Beyond mere contaminant mitigation, the current trajectory of environmental biotechnology emphasizes integrated solutions that provide auxiliary benefits.

Microbial Fuel Cell (MFC) technology exemplifies this shift, constituting a bioelectrochemical system designed to convert the chemical energy stored in organic matter—in this instance, hydrocarbon pollutants—directly into electrical energy via microbial metabolism (Logan *et al*., 2006). MFCs offer the dual advantage of remediating contaminated matrices while simultaneously generating sustainable, low-power electricity, effectively transforming waste into a valuable resource. The performance of an MFC is intrinsically linked to the electrogenic capacity of the microorganism used, specifically its proficiency in transferring electrons to the anode. A bacterium capable of degrading complex hydrocarbons, such as *E. profundum*, is thus an excellent candidate for serving as both the bioremediation agent and the power generating ability within an MFC system.

This research details the isolation, comprehensive biochemical, and molecular identification of the isolate. Crucially, it provides a rigorous quantification of its bitumen degradation efficacy via Gravimetric and Gas Chromatography-Mass Spectrometry (GC-MS) analysis, elucidating the specific metabolic transformations of the complex hydrocarbons. Finally, the study explores the practical application of this isolate within a constructed dual-chamber MFC, evaluating its potential for bitumen cleanup with the emerging objective of bioelectricity generation. The results aim to establish *E. profundum* as a versatile, indigenous bioconversion platform pertinent to addressing complex petrochemical waste challenges.

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.

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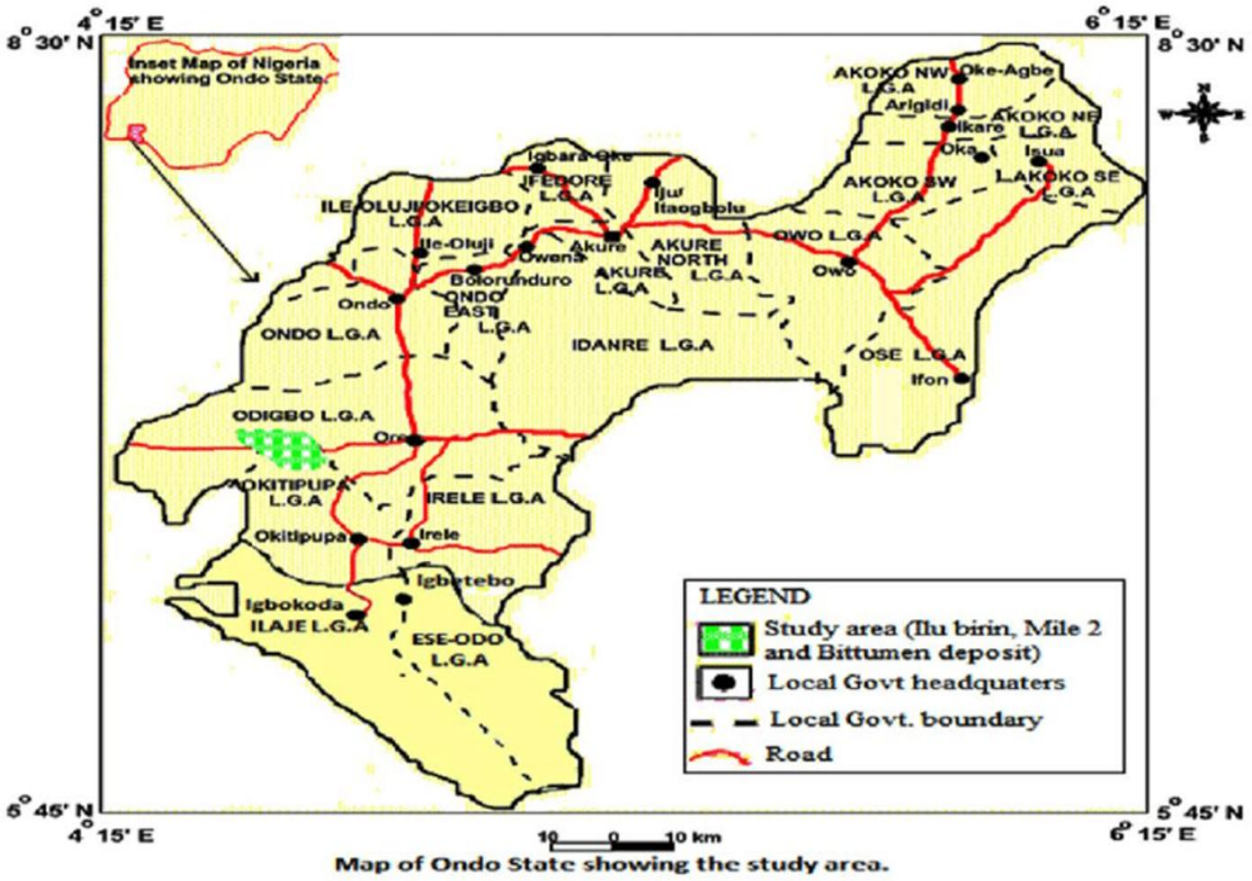

Figure 1: Sampling Site: Agbabu, Odigbo L.G.A., Ondo State (Olowomofe *et al*., 2019)

Water Samples

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

Sediment Samples

Sediment samples were also collected from the three sampling stations using a sterile auger. Samples were taken at 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 *Exiguobacterium profundum*

Culture Media Preparation

For the isolation of *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 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

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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 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 Isolate IID

Genomic DNA Isolation

Isolate IID was molecularly characterized by analyzing the 16S conserved region of the bacteria. The genomic DNA of the overnight grown culture of IID was 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, 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°C; 30 seconds), 32 cycles of denaturation (94°C; 30 seconds), annealing (45°C; 55 Seconds), initial extension (68°C; 60 seconds), final extension (68°C; 7 minutes) followed by holding (4-8°C). 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).

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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 (%) $= [(Initial\ oil\ content - Residual\ oil\ content) / Initial\ oil\ content] \times 100$

GC-MS Analysis

For the GC-MS analysis of soil and water samples, the process began with sample collection, where 50 g of soil and 100 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 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).

Microbial Fuel Cell (MFC) Construction

Construction of Cathode and Anode Chamber

Plastic containers (1.5 liter) served as the anode and cathode chamber, a hole was drilled at the sides of the container for fitting PVC pipe with an electric soldering iron, also a hole was drilled on the lid of the containers for passage of copper wire. Wax gum was used to seal the edges of contact between the holes at the sides of the chambers. The setup was tested for leakage by pouring distilled water into the chambers and observed for a day after which the distilled water was disposed.

Preparation of Salt Bridge

The salt bridge was prepared using 5% Sodium Chloride (NaCl) and 10% agar-agar. Five grams of NaCl was weighed into an empty pot with twenty grams of agar-agar. 150 ml of distilled water was added and boiled to dissolve the agar-agar. The agar was allowed to cool down to room temperature before pouring into the Polyvinyl Chloride (PVC) pipes of about fifteen centimeter long and four centimeter in diameter, were covered at one end with a nylon and rubber bands, once the pipes were filled, they were left to stabilize and solidify for about 10minutes.

Preparation of Electrodes

Electrodes was collected from dismantled batteries, the battery electrodes were sterilized using 95% ethyl alcohol, naked coppers wires were firmly wrapped around the electrodes.

Coupling of MFC

The set up was coupled by joining the two chambers together using the salt bridge which serve as membrane. The anode and cathode chambers, wires and electrodes were cleaned with 95% ethyl alcohol and passed under UV light to rule out any form of contaminants. The bitumen sample was equally sterilized under UV light. 1200 ml of mineral salt medium, 200 ml of bitumen and 100 ml of 24 hours old culture (*Exiguobacterium profundum*) made up the anodic chamber. The cathodic chamber contained Potassium permanganate solution which was prepared by dissolving 23.7 gram in 1500 ml of distilled water and in the second cathode chamber. The electrodes were carefully dipped into the chambers with the wires passing through the hole on the lids, a multi-meter was connected to the anode and copper wires was set for measurement of direct current and voltage. The initial reading was recorded and subsequent readings were taken 6 hours apart at morning, afternoon and evening for fourteen days.

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RESULTS AND DISCUSSION

Table 1: Biochemical identification of isolates IID

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

Keys: + = positive, - = negative

Table 1 outlines the biochemical characteristics of isolate IID, confirming its identity as *Exiguobacterium profundum*. The strain displayed positive catalase and citrate utilization capabilities which are indicative of its metabolic versatility. These traits position *E. profundum* as a suitable candidate for bioremediation applications (Manon *et al*., 2015).

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Table 2: Molecular Identity of isolates IID

Isolate Code	Molecular Identity	Accession number
IID	*Exiguobacterium profundum*	PP278055

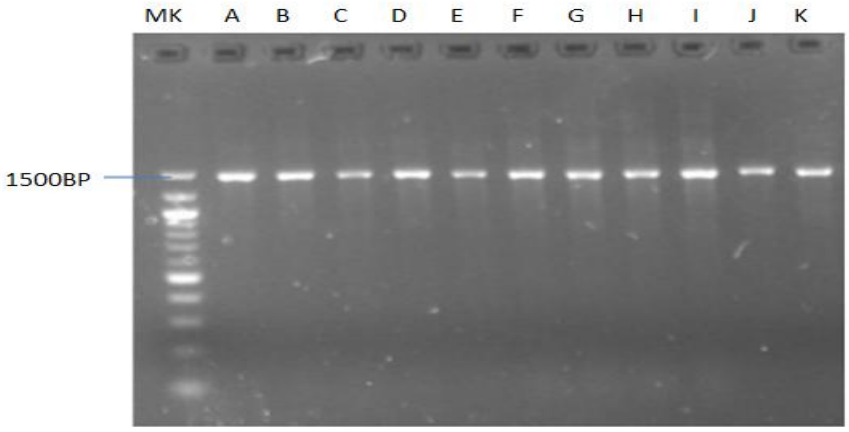

Plate. 1: Agarose gel electrophorogram of PCR reaction for DNA extracted from *Exiguobacterium profundum*

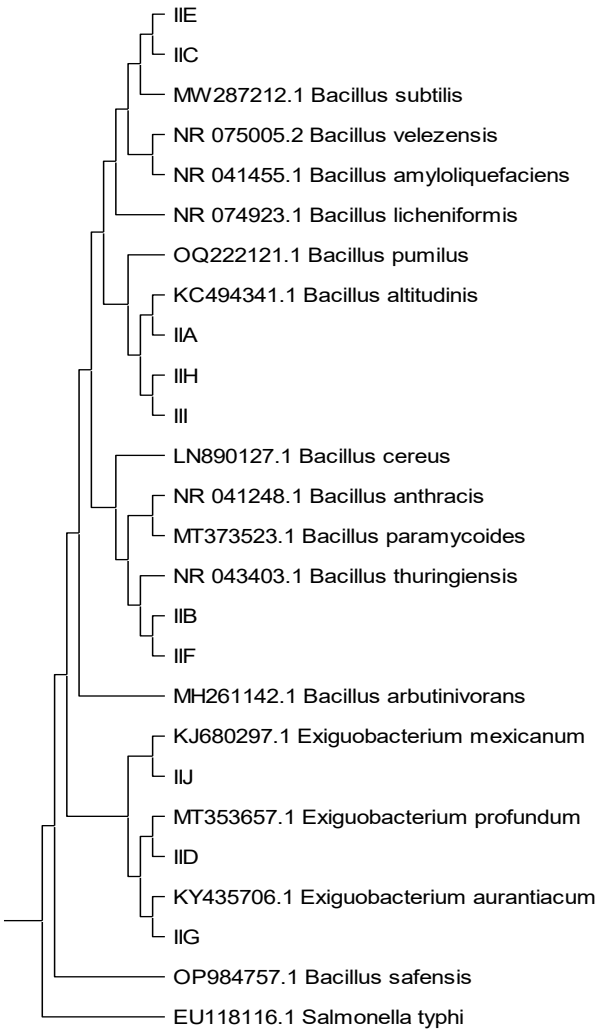

Fig. 2: Phylogeny tree of isolate IID

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AACCTACCGGAGGCACCCGTGGGGAATCTTCCCCAATGGACGAAAGTCTGATGGAGCAACCCCG
CGTGAACGATGAAGGCTTTCGGGTCGTAAAGTTCTGTTGTAAGGGAAGAACAAGTGCCGCAGGC
AATGGCGGCACCTTGACGGTACCTTGCGAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGG
TAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGCCTCTTA
AGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGCCATTGGAAACTGGGAGGCTTGAGTAT
AGGAGAGAAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGT
GGCGAAGGCGACTCTTTGGCCTATAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGA
TTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAGGTGTTGGAGGGTTTCCGCCCTTC
AGTGCTGAAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGGCTGAAACTCAAA
GGAATTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCAAAGCAACGCGAAGAAC
CTTACCAACTCTTGACATCCCCCTGACCGGTACAGAGATGTACCTTCCCCTTCGGGGGCAGGGGT
GACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGC
GCAACCCTTGTCCTTAGTTGCCAGCATTTGGTTGGGCACTCTAGGGAGACTGCCGGTGACAAACC
GGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGAGTTGGGCTACACACGTGCTAC
AATGGACGGTACAAAGGGCAGCGAAGCCGCGAGGTGGAGCCAATCCCAGAAAGCCGTTCTCAGT
TCGGATTGCAGGCTGCAACTCGCCTGCATGAAGTCGGAATCGCTAGTAATCGCAGGTCAGCATAC
TGCGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCACGAGAGTTTGCAACACCC
GAAGTCGGTGAGGTAACCGTAAG

Fig. 3: DNA Sequence of *Exiguobacterium profundum*

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 *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

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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

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GC-MS identified several degradation products, including decanoic acid and hexadecanoic acid (Table 4). The transition from complex hydrocarbons to simpler acids suggests a successful biodegradation pathway, aligning with discoveries from previous studies (Huang *et al.,* 2015).

Bioremediation efficiency

Table 6: Percentage weight loss of bitumen after bioremediation

Isolates	% weight loss
IID (*Exiguobacterium profundum*)	36.2

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. The gravimetric data demonstrated that the presence of *E. profundum* resulted in a significant reduction of the bitumen mass, achieving a 36.2% weight loss of the initial content after 30days (Table 6). This result attests to the strain's potent enzymatic system and its capability to initiate the breakdown of the highly recalcitrant heavy-weight hydrocarbon fractions characteristic of bitumen (Diallo *et al*., 2021).

Bioelectricity Generation Using Potassium Permanganate Mediator

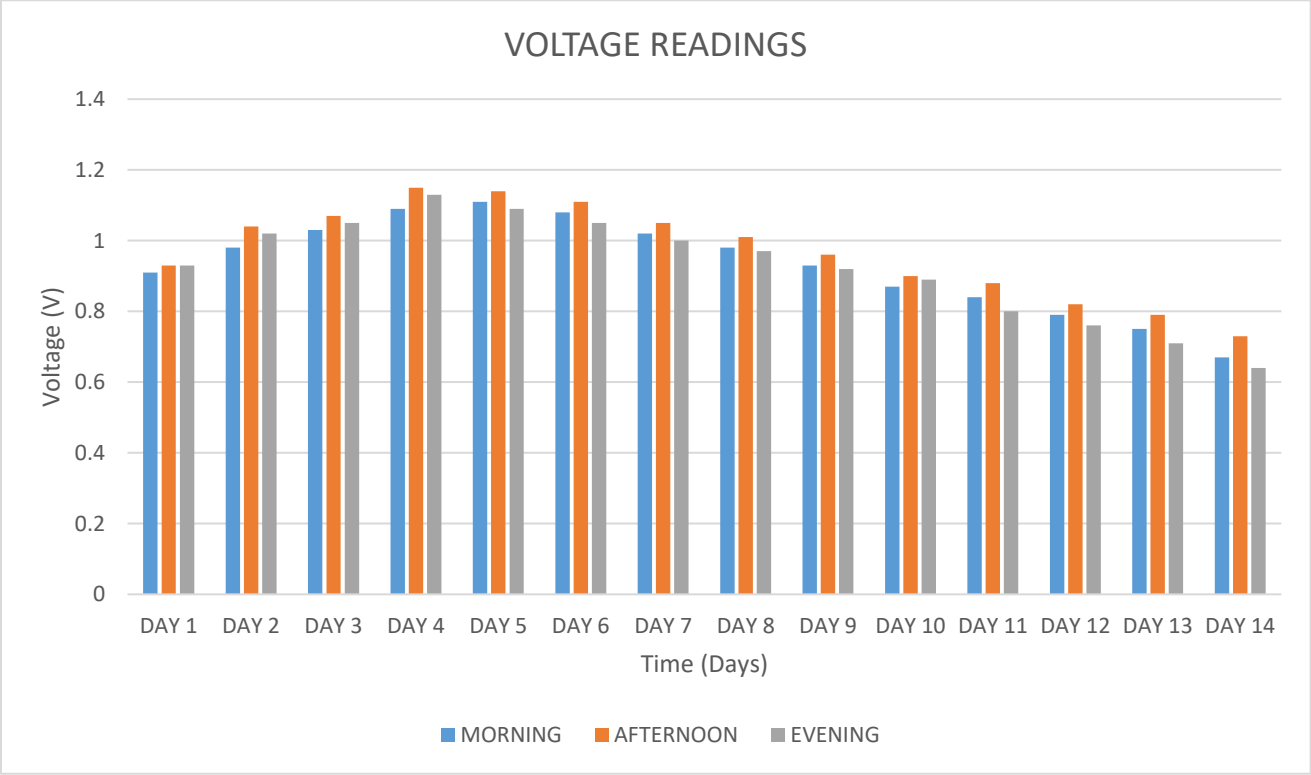

Fig. 3: Voltage readings

The average voltage of around 0.91 V to 0.93 V on Day 1 is quite competitive, nearing the higher end of the theoretical range. This suggests that the microbial fuel cell is operating effectively at the start of its experiment. Initially, the voltage readings fluctuate but overall exhibit a declining trend. The maximum voltage starts at 1.11 V in the morning of Day 5 and decreases to 0.67 V by Day 14. This decline could suggest a decrease in microbial activity or efficiency in converting substrates to current over time, indicating a potential limitation in the cell's performance or nutrient depletion. The steady decrease in voltage could point to reduced microbial activity or the declining efficiency of $KMnO_4$ as a mediator over time. Other factors such as accumulation of metabolic byproducts, depletion of nutrients (like the organic matter consumed), or unfavorable pH levels can potentially impede microbial function (Akujobi *et al.,* 2017).

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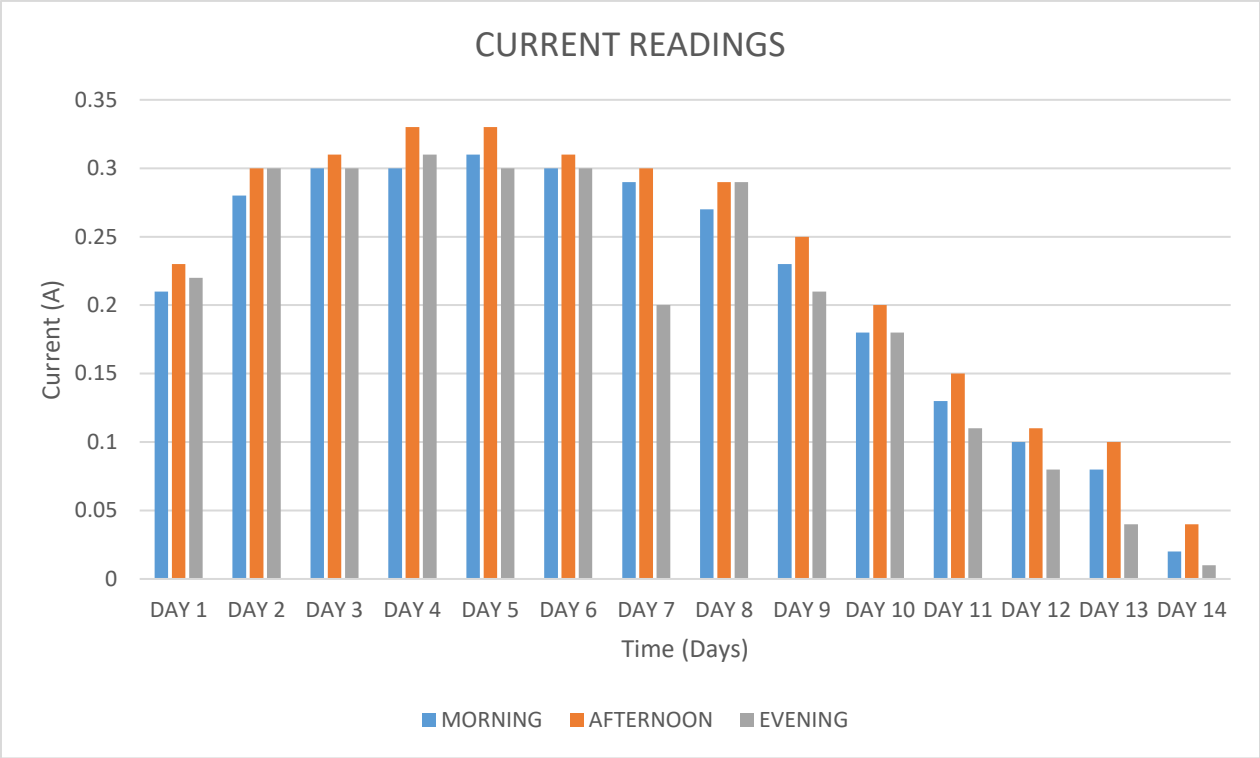

Fig. 4 : Current readings

Similar to voltage, current readings are also recorded in the morning, afternoon, and evening.

Current values reveal a more significant drop noticeable from Day 7 onward, indicating how the microbial activity influenced the production of electricity. The readings start with currents around 0.31 A on Day 5, but by Day 14, they plummet to 0.02 A. This significant drop suggests that the microorganisms may be losing their ability to generate current due to the reduced availability of active microbes or less effective electron transfer mechanisms, substrate depletion or changing environmental conditions affecting microbial metabolism. This agrees with report from Akujobi *et al.,* 2017. This observation supports the idea that the organism can utilize organic matter as a substrate while generating electricity, a characteristic that makes it promising for both bioremediation and bioelectricity applications (Santoro *et al*., 2017).

CONCLUSION

The investigation successfully isolated, identified, and characterized *Exiguobacterium profundum* (IID) from Agbabu bitumen environment. The results unequivocally establish the strain's utility as a pivotal component for two distinct, yet technologically integrated, environmental solutions. First, the organism demonstrated a highly effective bioremediation capability, achieving a 36.2% removal rate of complex bitumen within a 30-day period. Analysis via GC-MS provided definitive evidence of metabolic activity, showing the conversion of long-chain hydrocarbons into less hazardous, simpler organic acids and aldehydes. This confirms the activation of pathways necessary for the remediation of heavy petrochemical waste. Second, the successful integration of *E. profundum* into a dual-chamber Microbial Fuel Cell system highlights its potential for a resource-positive remediation paradigm.

By forcing the metabolic discharge of electrons to an external electrode, the system offers a pathway to convert the chemical energy encapsulated in bitumen into usable bioelectricity. The strategic incorporation of KMnO4 as an electron acceptor suggests a promising strategy for optimizing the efficiency and power output of this bioconversion process. The utilization of an indigenous, high-performance strain like *E. profundum*, adapted to the local environmental stressors, significantly enhances its potential for successful field deployment. The data strongly shows this isolate as a versatile organism capable of providing both robust environmental decontamination and sustainable energy recovery, thereby offering a viable and economically attractive strategy for managing complex petrochemical pollution.

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