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Characterization and Application of Biopolymer Producing Bacteria for Enhanced Oil Recovery

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ABSTRACT: *The objective of this research is to isolate and identify hydrocarbon-degrading bacteria for biopolymer synthesis and application in the augmentation of Nigerian heavy crude oil recovery. MEOR refers to the process of injecting either indigenous or non-indigenous microbes into hydrocarbon reserves. Injecting microorganisms with nutritional broth facilitate the formation of essential metabolites such as biosurfactants, biopolymers, and gases, resulting in decreased interfacial tension, viscosity modification, and mobility control. It is environmentally friendly, less expensive to implement, and requires minimal or no changes to the existing infrastructure. A soil sample from a hydrocarbon-contaminated site in Ogoniland was collected and sent to a laboratory for physicochemical and microbiological investigation. Bacillus sp, Pseudomonas sp, and Klebsiella sp were biochemically identified after screening three isolates for biopolymer production using Sudan black solution. To assess the ideal growth and biopolymer synthesis capability under reservoir conditions, a variety of pH, temperature, salinity, carbon, and nitrogen nutrition sources were applied to selected microorganisms. Peptone is the optimal nitrogen source for Bacillus sp, glucose is the optimal carbon source for Bacillus sp, and glycerol is the optimal carbon source for Pseudomonas sp and Klebsiella sp, as indicated by the results. In addition, the following are the ideal parameter ranges for the three microorganisms: pH 7–8, a temperature range between 25 and 350 degrees Celsius, and a salinity range between 0.5 and 5% are all desirable conditions for a body of water. After inoculation with microorganisms and the optimum nutrient source, an additional recovery range of 18.33% to 29.09% of the pore capacity was achieved. The post-recovery analysis uncovered a remarkable transformation of heavy crude to light hydrocarbon components by an average of 20.33 percent with glucose and 97.27 percent with peptone.*

KEYWORD: *Bacillus* sp, *Pseudomonas* sp and *Klebsiella* sp, biochemical characterization, microorganisms, biosurfactant, tertiary recovery, physicochemical and microbiological analyses.

INTRODUCTION

Currently, there is a global trend toward eliminating hydrocarbons as an energy source, and the bulk of the world's energy demands are met by oil and gas. Consequently, there is a push to meet the energy demands of the world's growing population. Hydrocarbon is a non-renewable energy source; hence it is difficult to extract the hydrocarbon content from reservoirs that have previously been discovered. The three stages of hydrocarbon recovery are primary, secondary,

and tertiary. The total recovery from these three techniques is insufficient to bring the full reservoir fluid to the surface. The primary recovery stage is only capable of recovering 20% to 30% of the reservoir fluid (Saleem et al, 2011) by utilizing the reservoir's natural energy or installing an artificial lift mechanism.

Secondary recovery techniques involving the injection of gas or water can recover up to forty percent of the oil that was originally there (Saleem et al, 2011). This method is used to increase the quantity of oil collected after the initial step. When water is injected at a specified rate into a reservoir, the water has a tendency to bypass some oil, known as residual oil, or to cause what is known as viscous fingering, resulting in the operators leaving a significant amount of oil in the pore spaces for various reasons (Ivan and Rafael, 2007).

A tertiary recovery technology known as enhanced oil recovery (EOR) is utilized to extract oil from the pore space after the primary and secondary recovery stages. This technique consists of chemical injection, cyclic steam injection or in-situ combustion, miscible gas flooding, form injection, and a promising microbial injection which is the subject of this research. The selected microorganisms' ability to recover heavy oil will be examined.

Ijeoma et al. (2016) isolated, characterized, and optimized biosurfactant-producing bacteria from the hydrocarbon-contaminated soil of Ogoniland. Ekpo and Udofia (2008) successfully characterized the large microbial communities present in the Qua Iboe Terminal's crude oil sludge environs. Ahmed et al. (2014) discovered and described twenty-three unique microbial colonies for the generation of biosurfactants in the Saudi Arabia region.
While Mandal et al. (2013) focused on the extraction, purification, and characterization of several antimicrobial lipopeptides and biosurfactants produced by Citrobacter and Enterobacter, others seek novel biosurfactants and compounds with potential applications in petroleum engineering (Youssef et al, 2004; Duarte et al, 2014). Nmegbu and Akpa (2014) investigated the viability of increased oil recovery utilizing gas-producing microorganisms such as Clostridium, Desulfovibrio, Pseudomonas, and methanogens. Therefore, the focus of this work is on microorganisms that create biopolymers for use in EOR. Several researchers, including the following, have made substantial contributions to MEOR approaches:

Ikporo and Okotie (2014) conducted a laboratory study employing Bacillus and Pseudomonas species to investigate the potential of MEOR for light Nigerian crude oil. Their data suggested that microbial flooding reduced residual oil saturation by an average of 23%. Abdullah et al. (2015) conducted the first experimental study on microbially enhanced heavy crude oil recovery by biodegradation using bacterial isolates from an Omani oil field. This approach resulted in the additional recovery of 16% of the oil that was initially present.

Gudia et al. (2012) cultivated 58 identified strains of bacteria isolated from crude oil samples on a mineral medium with a pH of 7.0 formulated by Raymond. Bacillus subtilis, a microbe that creates biosurfactants, was isolated from five of these strains during an experiment done in a sand-filled chamber at a temperature of 40°C under anaerobic conditions. After waterflooding, the IFT value was reduced from 66.4 to 30 mN/m, with a recovery range of 19.8 to 35% of the trapped oil, whereas AlSulaimani et al. (2012) observed a recovery of 23% after conventional water injection and an optimal recovery of 50% of the residual oil was achieved using a 50-50 ratio of biosurfactants produced by Bacillus subtilis and ethoxylated sulphonates,

respectively. Ruixia and Anhuai (2009) conducted a laboratory study on the biodegradation of three heavy oils at the Shengli oil field in East China using a halophilic bacterial strain.

MATERIALS AND METHODS

Materials required

- Soil samples
- Core samples
- Heavy crude sample
- Distilled water
- Mineral salt media (MSM)
- Aluminium foil
- Syringe
- Methylated spirit
- Toluene
- Industrial Salt (sodium chloride)
- Clinical cloves

Equipment Used

- Analytical weighing balance
- Turbid meter and UV spectrometer
- Inoculating loop
- Measuring cylinder
- Conical flask
- Viscometer
- Petri dish
- Bunsen burner
- Sieve and pH meter
- Core flooding equipment
- Rheometer
- Autoclaving machine

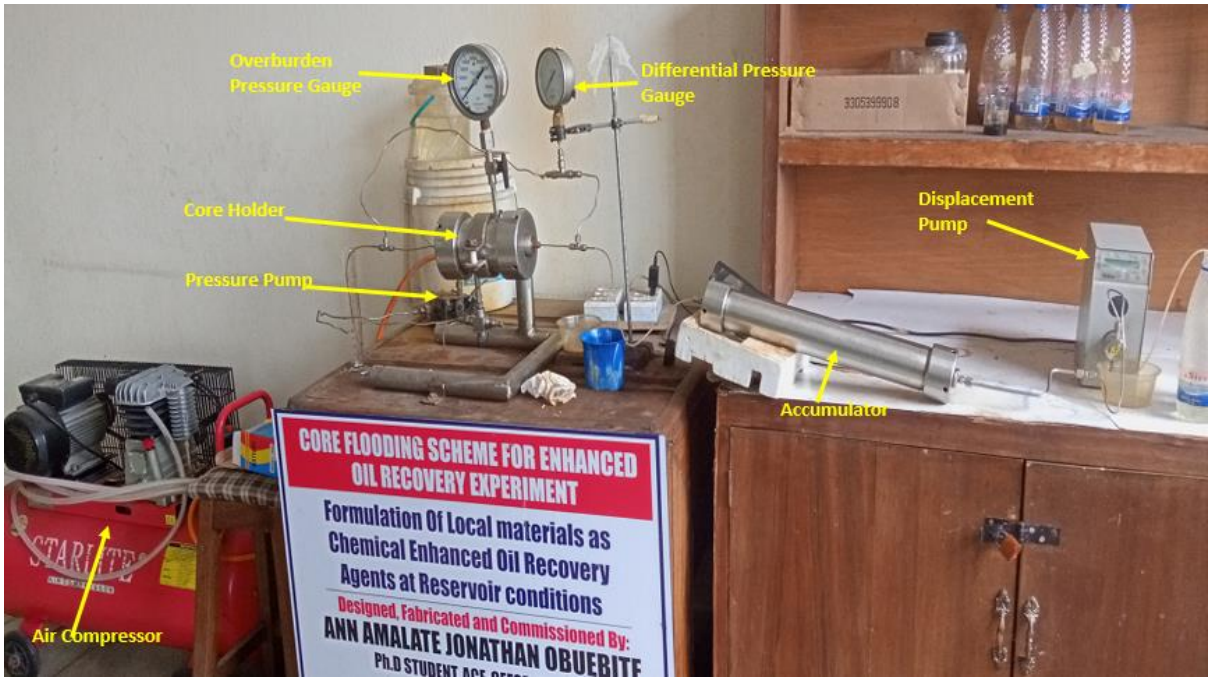

Figure 1: Core flooding experimental setup

METHODS

Hydrocarbon Contaminated Soils

Hydrocarbon-contaminated soil samples were collected from Gio, Tai Local Government Area (LGA) in Ogoniland, Rivers State. These samples were transferred into a polythene bag and

transported to the laboratory via ice pack for physicochemical and microbiological analyses. The samples were collected from five different points between the depths of 0 to 15 cm.

Sample Preparation

The preparation of the soil sample, derail dilution to determine the total viable cell count, preparation of the nutrient broth for the bacteria growth and biosurfactant product, brine and core sample preparation are presented in subsections below:

Processing of soil sample

Before using the soil samples for the various microbiological studies, the procedure outlined by Suja et al. was modified and applied to the soil samples (2014). In summary, the samples were dried in an oven at 40^{o}C for three hours (3 hrs), sieved using a 2-millimeter (mm) mesh sieve to eliminate undesired debris or particles, and then refrigerated at 4^{o}C before use.

Serial dilution

To perform ten-fold serial dilution, 9 mL of normal saline (0.85% NaCl w/v in distilled water) was first distributed into clean 25 mL (28x85 mm) McCartney bottles (Hurst, Australia). The bottles were corked properly, sterilized in an autoclave at 121 ^{o}C (15 psi) for 15 min and allowed to cool at room temperature. One gramme of each of the processed soil samples was added to 10 mL of sterile normal saline to make stock solution. From this stock solution, 1 mL was pipetted into a corresponding McCartney bottle containing 9 mL sterile normal saline to make 10^{-1} and thereafter 10^{-2}, 10^{-3}, 10^{-4}, $10^{-5}$and 10^{-6} dilutions.

Isolation of hydrocarbon-degrading bacteria by enrichment method

The processed soil samples were used for the isolation of hydrocarbon-degrading bacteria and comprised seven soil samples obtained from different locations and labelled as A, B, C, and D. The method described by Mittal and Singh (2009) with slight modification was used. In brief, 10 g of the processed soil was dissolved in 250 mL Erlenmeyer's flask, containing 100 mL of sterilized normal saline. The flask was votexed at maximum speed for 2 min and the suspension allowed settling for 5 min. A volume of 5 mL of the supernatant was used to inoculate another separate flask containing 100 mL Bushnell Haas Broth (BHB) (containing in g/L: 0.2 $MgSO_4.7H_20$; 0.02 $CaCl_2.2H_2O$; 1 KH_2PO_4; 1 K_2HPO_4; 1 NH_4NO_3; 0.05 $FeCl_3$; nystatin- 0.1g; and pH 7.0) supplemented with crude oil (1%, v/v) as the sole carbon and energy source. Tween 80 (0.05% v/v) was added to the broth to enhance hydrocarbon degradation. The procedure was repeated for each of the soil samples and the set-up performed in duplicate. The flasks were incubated in a rotary shaker incubator at 150 rpm for 7 days. At the end of 7 days, successive sub-culturing were done by transferring 5 mL of BHB culture into fresh BHB medium supplemented with crude oil (1%, v/v). The sub-culturing ensured isolation of only oil-tolerant and oil-degrading bacteria.

After three sub-culturing stages, 1 mL of the broth was pipetted aseptically and serially diluted to make $10^{-1}$to 10^{-6} dilutions. The 10^{-3}, 10^{-5}, and 10^{-6} dilutions were plated out on a freshly prepared Bushnell Haas agar plates supplemented with crude oil (1% v/v). The inoculated plates were incubated for 4 days at 30 ^{o}C. Discrete colonies on the plates were picked and purified by repeated streaking on Bushnell Haas Agar supplemented with crude oil (1%, v/v). The pure isolates were further purified on nutrient agar and stored in Bushnell Haas Agar slants supplemented with 1% (v/v) crude oil.

Screening of isolated bacteria for biopolymer production

Bacterial isolates were cultured for 2 days at 37 °C in Yeast Mold (YM) agar. From each, a thin film of cells was smeared on a clean, sterile glass slide, heat-fixed and stained with 0.3% Sudan black solution for 10 minutes, treated with ethanol for few seconds and washed gently with distilled water. As a contrast stain, the film was covered with red Safranin stain (0.5%) for 30 sec and gently rinsed with water to remove excess stain. Finally, air-dried bacterial film was examined under microscope using an oil immersion lens. The isolates were characterized as biopolymer positive (+ve) or biopolymer negative (-ve) based on the Sudan Black staining of the bacterial cells. Here the biopolymer positive (+ve) cells picked up the Sudan black stain and appeared dark (blue black inclusions within the cells) when observed under the light microscope, whereas negative cells were stained by the counter stain (safranin) only and they appeared pink.

Quantitative determination of biopolymer produced by bacterial isolates

A standard inoculum was prepared by inoculating a loopful of cell from the YM plates into 10 ml of YM broth and incubated at 37ºC for 24 h. Two percent (v/v) of each of the broth cultures was inoculated into the fermentation medium containing (glucose – 20 g/l, ammonium sulphate $(NH_4)_2SO_4$ – 2.0 g/l, potassium dihydrogen phosphate (KH_2PO_4) – 13.3 g/l, Magnesium sulphate ($MgSO_4.7H_2O$) – 1.2 g/l, Citric acid – 1.7 g/L, Manganese sulphate ($MnSO_4$) – 0.4g/l, ferrous sulphate ($FeSO_4$. $7H_2O$) – 1.0 g/l, $CoCl_2$. $6H_2O$ – 0.2 g/l, zinc chloride ($ZnCl_2$) – 0.2 g/l which was incubated at 37ºC and 120 rpm for 72 h. the pH of the medium was maintained at 7. At the end of the incubation period, extraction of biopolymer was done using sodium hypochlorite extraction method. Fifty millilitres of incubated fermentation medium was taken and centrifuged at 6000 rpm for 15 mins and the supernatant was discarded.

The cell pellets were suspended in 1.0M of sodium hypochlorite and incubated for 2 h at 37ºC. This mixture was centrifuged at 6000 rpm for 15 mins and the supernatant was discarded. The sediment was washed twice with distilled water and centrifuged. The polymer granule was washed with a mixture of ethanol and acetone (2:1). The polymer was finally dissolved in hot chloroform and was evaporated by air drying to yield dry powder of biopolymer. The yield of the biopolymer was expressed in g/l.

Determination of biomass (dry weight)

Broth cultures (50 ml) after incubation for three days, was again centrifuged at 6000 rpm for 15 mins and the supernatant was discarded. The cell pellet was washed with water and finally allowed to dry overnight at 60ºC till constant weight (g/l) was attained. The percent yield of the polymer was expressed as follow:

$$Biopolymer\ yield\ (\%) = \frac{biopolymer\ (dry\ weight)}{biomass\ (dry\ weight)} * 100 \qquad (1)$$

Identification of bacterial isolates

This was done following the method of identification in accordance with Cheesbrough (2004), using morphological characteristics and biochemical tests such as:

i. Gram Stain
ii. Motility Test
iii. Catalase Test
iv. Oxidase Test
v. Indole Test

Optimization for Nutritional and Environmental Factors

The optimization study was carried out on the following parameters with range of values to ascertain their effect on the growth and biopolymer production of the selected microbes:

i. pH (5, 6, 7, 8, 9, 10 and 11)
ii. Temperatures (25, 30, 35, 40, and 45 ^{o}C)
iii. Carbon sources (dextrose, fructose, glucose, glycerol, starch and sucrose)
iv. Nitrogen sources (asparagine, NH_4NO_3, peptone, urea and yeast extract)
v. Salinity (0.5%, 1%, 5%, 9%, 13%, and 15% (w/v).
vi. Inoculum size (0.25, 0.5, 1, 2, 3 and 4 % (v/v))

4 Results and Discussion

The biochemical characterization of the selected microbes is presented in Table 1

Table 1: Identification of bacteria isolates

Sample	Gram reaction	Shape	Catalase Test	Oxidase Test	Motility Test	Indole Test	Glucose	Glucose	Lactose	Acid	Gas	H_2S	Organisms Identified
SO-12	-	Rod	+	-	+	-	+	+	+	+	+	-	*Bacillus* sp
SO-33	-	Rod	-	+	-	+	+	+	+	+	-	-	*Pseudomonas* sp
SO-34	+	Rod	+	+	+	-	+	+	+	+	+	+	*Klebsiella* sp

Screening of isolated bacteria for biopolymer production

The selected *Bacillus* sp, *Pseudomonas* sp and *Klebsiella* sp were cultured for 2 days at 37 °C in yeast mold (YM) agar to test for their ability to produce biopolymer. The isolates were characterized as biopolymer positive (+ve) or biopolymer negative (-ve) based on the Sudan Black staining of the bacterial cells. Here the biopolymer positive (+ve) cells picked up the Sudan black stain and appeared dark (blue-black inclusions within the cells) when observed under the light microscope, whereas negative cells were stained by the counter strain (safranin) only and they appeared pink. The result in Table 2, shows that *Bacillus* sp and *Pseudomonas* sp are good biopolymer producers while *Klebsiella* sp showed no dark colour, hence cannot produce biopolymer.

Table 2: Screening of isolates for biopolymer production

Sample	Bacterial Isolate	Staining with sudan black B
SO-12	*Bacillus* sp	+++
	Bacillus sp	++
	Bacillus sp	+++
	Pseudomonas sp	++
SO-33	*Pseudomonas* sp	+++
SO-34	*Klebsiella* sp	-
	Klebsiella sp	-

Key: *No dark colour* -, *light bluish-black* +, *moderate* ++, *good* +++

Enumeration of bacteria

To account for the number of pure culture *Bacillus* sp, *Pseudomonas* sp and *Klebsiella* sp injected per milliliter in the nutrient broth during the core flooding experiment, their viable cells (bacterial enumeration) were counted and are given below.

Microbes	Bacteria enumeration cfu/ml
Pseudomonas sp	$1.40 * 10^7$
Bacillus sp	$1.65 * 10^7$
Klebsiella sp	$1.10 * 10^7$
Consortium sp	$1.16 * 10^7$

Effect of Physicochemical and Nutrient Parameters on Biosurfactant Production

Temperature and pH

The optimum values obtained during the optimization study were employed in the formulation of the nutrient broth and core flooding experiment. Hence, the optimal pH range that favours the growth and production of biopolymer of *Bacillus* sp and *Pseudomonas* sp is 7 – 8, while 6 – 7 for *Klebsiella* sp as shown in Table 3. Therefore, the optimum pH value for *Bacillus* sp and *Klebsiella* sp is 7 while *Pseudomonas* sp is 8. The pH growth range corresponds to the result of Ruixia and Anhuai (2009). The result in Table 3 indicates a low growth and production of biopolymer and biosurfactant at lower and higher pH value with the three microbes. The effect of different incubation temperatures at 25, 30, 35, 40, 45 and 50^{o}C) on growth and biopolymer production after optimal pH had been determined. The bacteria were inoculated in a mineral

salt media supplemented with kerosene at the optimum pH for 3 days. The result in Table 3 shows an optimum incubation temperature of 30^{o}C for *bacillus, pseudomonas* and *Klebsiella* sp respectively which correspond with the result obtained by Sudhir (2015). The result shows that *Klebsiella* cannot withstand temperature of 50^{o}C.

Table 3: Effect of pH on the growth of hydrocarbon degrading bacteria

Temp. (^{O}C)	Bacillus *sp* OD at 600nm	Pseudomonas *sp* OD at 600nm	Klebsiella *sp* OD at 600nm	pH	Bacillus *sp* OD at 600nm	Pseudomonas *sp* OD at 600nm	Klebsiella *sp* OD at 600nm
25	0.408	0.387	0.168	5	0.182	0.171	0.089
30	0.436	0.424	0.214	6	0.199	0.179	0.107
35	0.411	0.463	0.206	7	0.329	0.421	0.196
40	0.206	0.301	0.186	8	0.376	0.482	0.141
45	0.179	0.271	0.162	9	0.353	0.433	0.159
50	0.164	0.265	0.085	10	0.358	0.321	0.103
				11	0.275	0.317	0.081

Nutrient Sources

The microorganisms can only create the appropriate metabolites if they are injected with the proper nutrients, such as carbon source, nitrogen source, etc. According to Table 4, five distinct carbon sources were utilized for the optimization evaluation in this investigation. The best carbon source for bacillus and pseudomonas was glucose, while the best carbon source for Klebsiella was glycerol. In the case of nitrogen nutrient, the results of this investigation indicate that peptone is the optimal source for all three isolated microorganisms. Thus, employed in the MEOR procedure.

Table 4: Effect of carbon source on the growth of hydrocarbon degrading bacteria

Carbon source	Bacillus *sp*	Pseudomonas *sp*	Klebsiella *sp*	Nitrogen source	Bacillus *sp*	Pseudomonas *sp*	Klebsiella *sp*
glycerol	1.708	1.84	0.718	Asparagine	1.691	1.683	0.785
dextrose	1.337	1.535	0.522	peptone	1.768	1.697	0.885
sucrose	1.098	1.003	0.582	Urea	1.682	1.576	0.818
fructose	1.513	1.011	0.547	yeast extract	1.37	1.581	0.417
glucose	1.759	1.502	0.705	ammonium nitrate	1.748	1.622	0.489
glycerol	1.708	1.84	0.718				

Salinity

The salinity of the hydrocarbon formation has a large effect on the growth and biosurfactant production for application in enhanced oil recovery. The result shows that as the salinity is increase, the growth and performance reduce as shown in Table 5. *Bacillus* cannot withstand

salinity beyond 5%, *Pseudomonas* can withstand up to 9% while *Klebsiella* is 1% as presented in Table 5.

Table 5: Effect of salinity on the growth of hydrocarbon degrading bacteria

Salinity (%)	Bacillus *sp* OD at 600nm	Pseudomonas *sp* OD at 600nm	Klebsiella *sp* OD at 600nm
0.5	0.767	0.673	0.131
1.0	0.713	0.602	0.110
5.0	0.753	0.584	0.063
9.0	0.531	0.428	0.061
13.0	0.455	0.367	0.041
15.0	0.502	0.235	0.044

Core Flooding Outcomes

The additional volume of oil recovered after the injection of microorganisms with their nutrient broth is depicted in Figures 2 as representing a maximum recovery of 27.13 percent of the pore volume with the consortium of Pseudomonas sp, Bacillus sp, and Klebsiella sp using glucose as the carbon source. Figure 3 depicts a maximum recovery of 24,81% of the pore volume with peptone as the nitrogen source. Pure cultures of Pseudomonas sp produced the lowest nitrogen and carbon results, with 17.05% and 15.50%, respectively. This compares to Tingshan et al. (2005), who found a maximum recovery of 35.7% in the Qinghai heavy oil field, and Abdullah et al. (2015), who found an additional 16% recovery of the initial oil in place from the MEOR process in the Omani oil field.

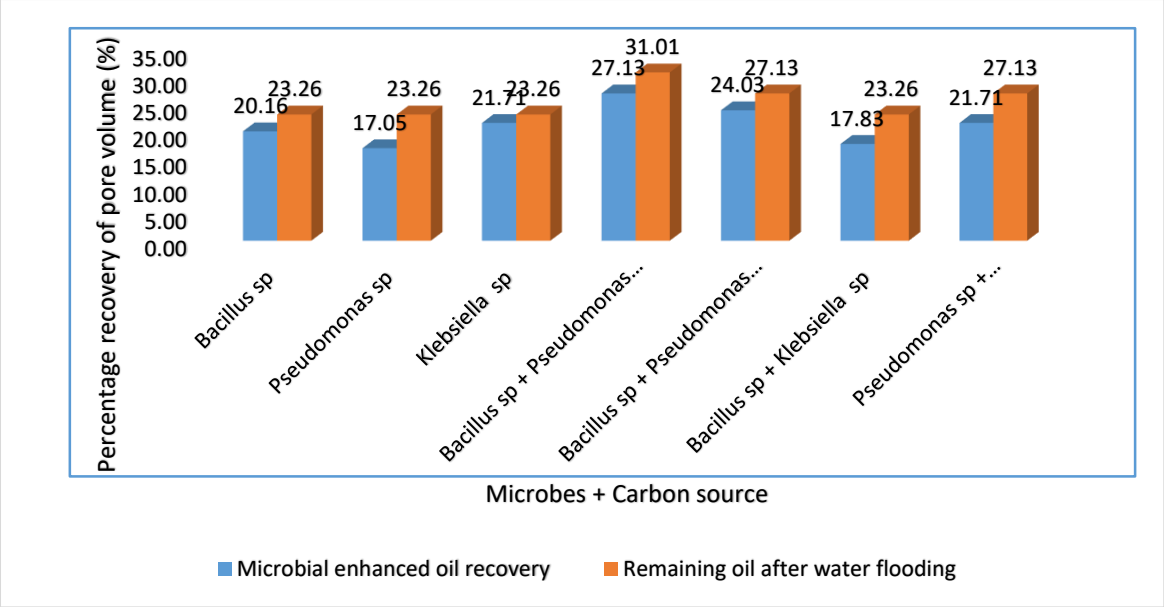

Figure 2: Percentage of pore volume of oil recovered after MEOR technique with carbon

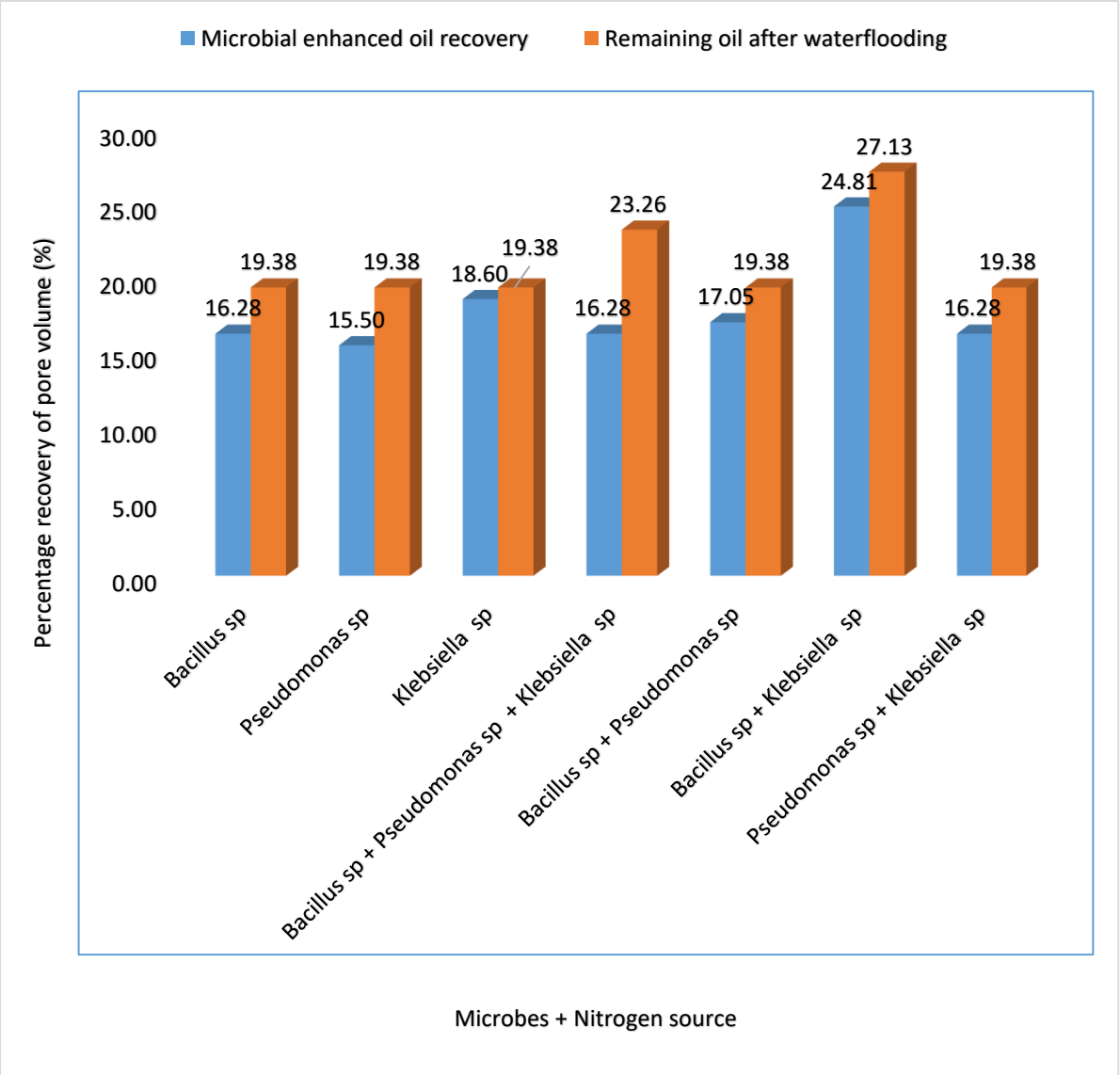

Figure 3: Percentage of pore volume of oil recovered after MEOR technique with nitrogen

Effect of Microbial Activities on the Oil Viscosity

A post-recovery analysis was conducted to determine the impact of microbial activity on the viscosity of heavy oil. The viscosity was measured with a rheometer R200 rotational viscometer at 500 revolutions per minute for thirty seconds. The outcome of the additional recovery indicates a significant biotransformation of heavy oil. This suggests that the majority of the heavier oil's carbon-rich fractions were converted into lighter hydrocarbon components. The initial viscosity (control) of the crude is 208 m.Pa.s, and the result achieved after inoculating the microorganisms with glucose and peptone as nutrition sources is shown in Table 6. Bacillus sp reduced the viscosity by 9.62 %, the least of all the pure and consortium bacteria, but Klebsiella sp lowered the viscosity of the heavy oil by 98.17 % using peptone. Pseudomonas sp produced the best results, at 25.48 %, when compared to Klebsiella sp and glucose. Klebsiella sp decrease with peptone nutrition was 98.17 %, followed by Bacillus sp reduction of 97.74 percent and Pseudomonas sp reduction of 96.16 %. Bacillus sp and Klebsiella sp reduced glucose by 32.69 % more than Klebsiella sp and Pseudomonas sp, which

reduced glucose by 97.93 %. This conclusion is consistent with the findings of Ruixia and Anhuai (2009) regarding the transformation of heavy oil in East China's Shengli oil field by a halophilic bacterial strain at a temperature of approximately 37°C and ideal pH range of 6.5 to 7.0. The results demonstrated a decrease in paraffin content of 4.06 %, 28.08 %, and 55.05 % in the three heavy oils employed in the experiment. Figure 4 demonstrates that, as a result, all experimental r uns with peptone nutrient produced considerably superior results than those with glucose foodstuff. Table 6: The effects of microbial activities on the physical's properties of crude oil.

SAMPLE TAG	M.H_2O (g)	M.C.OIL (g)	C. DENSITY (g/ml)	S.G.STD	API GRAVITY	VISCOSITY m.Pa.s
Control	27.65	26.55	1.062	0.9680	14.67	208
Bacillus + car	27.98	26.91	1.076	0.9690	14.53	188
Klebsiella + car	28.92	27.32	1.093	0.9519	17.15	155
Pseudomonas+ car	27.58	26.43	1.057	0.9655	15.050	170
Kleb+Bac + car	27.60	26.51	1.060	0.9677	14.72	150
Pseudo+ Bac + car	29.93	28.50	1.140	0.9594	15.99	140
Kleb+Pseudo + car	27.56	26.40	1.056	0.9658	15.05	184
Kleb+Bac+Pseudo + car	28.59	27.05	1.082	0.9533	14.25	173
Bac+Peptone	27.53	26.36	1.102	0.9647	15.18	4.7
Pseudo + Peptone	29.91	28.46	1.138	0.9594	15.99	8.0
Kleb + Peptone	27.88	26.42	1.057	0.9560	16.59	3.8
Bac +Pseud+pep	27.60	26.14	1.046	0.9549	16.67	5.6
Bac + Kleb+Pep	27.75	26.74	1.069	0.9708	14.26	7.1
Kleb+Pseud+pep	27.99	26.85	1.074	0.9665	14.90	4.3
Bac+Kleb+Pseod+pep	27.48	26.22	1.049	0.9613	15.69	6.3

The above, data are the result from experimental procedure according to American Society for Testing Materials (ASTM) D1217; Standard Test Method for Density, Relative Density (Specific Gravity) by Bingham Pycnometer and DIN/ISO 3219 System for Absolute viscosity.

Key:
Bac = Bacillus
Pseudo = Pseudomonas
Kleb = Klebsiella
Pep = Peptone

CONCLUSION

Bacillus sp, Pseudomonas sp, and Klebsiella sp were detected in a hydrocarbon-contaminated soil sample as unique and promising indigenous biopolymer-generating bacteria. The results reveal that salinity, temperature, pH, and nutrient sources all have an effect on the bacteria in

question. The MEOR technique increased oil recovery by 24.5 % using glucose broth and by 21.9 % using peptone broth. The post-recovery analysis uncovered a remarkable transformation of heavy crude to light hydrocarbon components by an average of 20.33 percent with glucose and 97.27 % with peptone.

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List of Abbreviations

Abbreviation	Meaning
pH	Potential of hydrogen
sp	Species
EOR	Enhanced oil recovery
MEOR	Microbial enhanced oil recovery
IFT	Interfacial tension
LGA	Local Government Area
BHB	Bushnell Haas Broth
YM	Yeast Mold

Availability of data and materials

The data and materials for this study are available in the manuscript submitted.

Competing interests

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Authors' contribution

Aruawamedor, Onome Christopher carried out the research methodology, involved in the insolation and screening of the microbes, performed the entire investigation, participated in the interpretation of the results, developed the manuscript and also revised the draft manuscript. Okotie Sylvester performed the conceptualization of the research idea, involved in the experiments and in the interpretation of the results, reviewed the draft manuscript and supervised the entire project. Both authors contributed to the discussion of the results and visualization of the final manuscript.

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