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Application of thermotolerant petroleum microbes at reservoir conditions for enhanced oil recovery

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ABSTRACT

Primary oil recovery is the first stage of hydrocarbon production in which a reservoir uses its natural energy to force hydrocarbon to its wellbore. Secondary oil recovery comes to play when hydrocarbons can no longer be further produced by natural means. The purpose of secondary recovery is to maintain reservoir pressure so as to displace hydrocarbons toward the wellbore. Both primary and secondary recovery processes cannot displace more than 50% of the available hydrocarbons in a reservoir. The remaining hydrocarbons are further recovered through Tertiary/Enhanced Oil Recovery techniques. According to literature, microbial enhanced oil recovery has been identified as a tertiary method used to improve the efficiency of hydrocarbon production from reservoirs. Microbial enhanced oil recovery is a feasible reservoir technology, which has not been widely used in the oil and gas industry owing to the attainment of the requisite reservoir conditions such as temperature within which microbes can thrive. Literature has shown that thermotolerant microbes can withstand optimum temperatures of 50–90°C, while deep and ultra-deep hydrocarbon reservoir temperatures are often above 100°C. This study identifies some isolated thermotolerant microbes from a sandstone reservoir that can withstand temperatures as high as 110°C via conventional methods and molecular analysis. The identified thermotolerant petroleum microbes: *Bacillus amyloliquefaciens* (A) and *Bacillus nealsonii* (B) were used to enhance oil recovery from a reservoir. The results showed that the microbial species A and B at a confined pressure of 3.0 MPa and temperature of 27°C, gave 46.4% and 48.6% oil recoveries, respectively, which is comparably higher than the value (26.9%) obtained for the water flooded samples. At temperatures of 80, 90, 100, 110 and 120°C, the oil recovery results show that the recovery factor (55.2–64.1%) of species B were higher compared to the range (46.7–57.5%) recorded for species A. At the onset of the core flooding experiments, there was an initial increment in oil recovery factor as the temperature increased from 80 to 110°C, whereas, it remained constant within 110–120°C. This trend coincides with the drop in the thermal resistance exhibited by the microbes when exposed to such conditions. The cumulative oil production from the commercial Eclipse simulation closely matched those of the experiment results, whereas, the slight difference can be attributed to the adjustment of the simulation input parameters. The experimental results show that species B can be used to enhance oil recovery at reservoir temperature conditions above 100°C.

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1. Introduction

Oil field development refers to the process of transporting liquid and gaseous hydrocarbons that have accumulated in a reservoir to producing wells. Oil is initially produced from reservoirs using the natural driving force of the reservoir (primary recovery) or by the

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application of other sources of energy in the reservoir (secondary oil production/flooding operations) once natural energy is depleted in the reservoir [1]. Currently, new but few drilling sites in mature fields are of commercial interest. In this regard, the residual oils remaining in these mature hydrocarbon reservoirs after primary and secondary oil recoveries, provide the opportunity to improve oil recovery via tertiary means; this process is known as microbial enhanced oil recovery (MEOR) [2].

Lazar et al. [3] highlighted the application of microbes in extracting residual oil from reservoir formations/porous media. The residual oil remaining in the depleted mature hydrocarbon reservoirs after primary and secondary oil recoveries makes it possible to carry out enhanced oil recovery via low cost and environmentally friendly advanced microbial enhanced oil recovery (MEOR) techniques [2]; this involves the deployment of ex-situ and in-situ metabolites. Maudgalya et al. [4] observed in their study that the temperature limit for most of the MEOR field trials are within 200 °F (which is less than 100°C). The global demand and consumption of hydrocarbon has altered the search and exploration of large fields to deep and ultra-deep environments, where the average temperature of the reservoir is often >200°F (i.e., >100°C). Most of the aerobic microorganisms are found in formations whose temperatures are within the range of 158°F (70°C) to 194°F (90°C) [5,6]. Microorganisms metabolize hydrocarbons at different rates, and according to Sivasankar and Govindarajan [7], microbes used for enhanced oil recovery processes must be able to survive the average reservoir conditions/temperature. Li et al. [8] and Junzhang et al. [9], showed the effect of high temperature oil reservoirs on microbes during oil recovery and justified the need to identify and isolate high thermophilic microbes that can be used for microbial enhanced oil recovery in deep and ultra-deep reservoirs after primary and secondary recovery processes.

MEOR also follows the basic principle of enlarging the inherent sweep efficiency in reservoirs, thus increasing the capillary number required for optimum oil recovery [10]. With the current uncertainties in oil prices, MEOR is a promising method, especially for uneconomical reservoirs, hence, microbial flooding has become a possible alternative for other enhanced oil recovery methods owing to its high success rates (i.e., up to 90%) [11,12]. MEOR is environmentally friendly and has no known negative impact on the environment. From literature, it has been noted that the successful application of MEOR is dependent on the hydrocarbon-bearing reservoir temperature [13]. Therefore, microbial technology has been identified as one of the future research areas with great potentials for improved oil recovery [14]. Temperature is one of the main environmental factors that govern and influence the development of microbial life [15]. Microbial communities in oil formations are dated back as the earth's most ancient biocenoses, which sank to great depths along with organic residues and biogenic sludge. Since only a very few species from this group of microorganisms have been isolated till date, there seems to be a large number of hyper thermophilic microbial species with unique properties awaiting discovery [16,17].

MEOR methods can be divided into two main groups: ex-situ MEOR, which involves the metabolites that use chemicals such as biosurfactants, biopolymers and emulsifiers to enhance their activities. Thus, the microorganisms are cultured outside the reservoir formation and then introduced into the formation in aqueous solutions [18]. For in-situ MEOR, the oil formation zone is the abode of microbiological activities that take place directly in the reservoir. Thus, this method is based on the microbial community of a specific reservoir, while the MEOR ex-situ method is based on the introduction of preselected microorganisms that are grown externally and pumped into the reservoir [19]. MEOR metabolites are produced from local or exogenous bacteria and are pumped into the reservoir [20]. Depending on the source of the strains, microbial flooding can also be divided into indigenous and exogenous microbial flooding [21]. In exogenous microbial flooding, suitable microbes are isolated under similar conditions but not in the reservoirs before being injected into the reservoir to increase oil production. In indigenous microbial flooding, the microbes are grown amidst the residual oil as carbon source which serves as the active ingredient present in the reservoir alongside air, inorganic salt, phosphorus and nitrogen which accompany the injected water [22].

Unfortunately, not all indigenous microbes can be used to recovery oil. The application of microbial technology to oil reserves through reservoir engineering designs, is often considered to uncertain due to the lack of understanding of the underlying mechanisms that stimulate microbial activity. Another challenge associated with MEOR is that most of the microbes used are sourced from hydrocarbon polluted sites on the surface, and as such, cannot withstand the subsurface reservoir temperature when used for MEOR [23–27]. To better elucidate the existence of petroleum thermotolerant microbes that can be used for enhanced oil recovery at reservoir temperature conditions, this study successfully isolated two petroleum thermotolerant microbes from a sandstone reservoir by culturing and categorizing them using gene sequence analysis. The isolated microbes were further identified by combining traditional and molecular analysis. The biochemical properties of the microbes were characterized while their abilities to survive reservoir thermal-conditions were also verified. The Haq et al. [23] commercially modified Eclipse simulator was adopted in simulating the MEOR core-flooding experiments using two microbes. Thus, this study investigates two thermotolerant petroleum microbes that can be used for ex-situ microbial enhanced oil recovery within reservoir thermal-conditions. MEOR is considered the most economical method for enhanced oil recovery, which in turn maximizes the profitability of the production process [28–33], thus, a good understanding of the underlying mechanisms in enhancing oil recovery is required for full implementation [24,36].

2. Methodology

2.1. Collection of reservoir sand from the production platform

The reservoir sandstone sample was collected from an offshore platform in the Gulf of Guinea. The oil well was drilled and completed as a single oil source which has been producing crude oil over the last seven years. According to Okoro et al. [37], the lithology obtained from this location describes the lithofacies as comprising of massive mudstone, bedded muddy heterolith, shelly muddy sandstone and pebbly sandstone. Most of the reservoirs in the area are fairly consolidated sandstones. The produced sandstone from the reservoir located at about 13, 657 ft, was collected from the in-situ treatment facility at the surface. The sample is of fairly uniform size and was stored in a high-density plastic keg, marked with an indelible permanent marker before it was delivered to Covenant University Biochemistry Molecular Laboratory for analysis.

2.2. Media preparation

The aseptic techniques adopted in this study include disinfection of work surface in the microbiology lab with 70% ethanol, washing of all glassware and apparatus to be used, draining and sterilization of the apparatus used in an oven at 180°C for 2 h; these steps prevent contamination of cultures from foreign bacteria which are inherent in the environment, thus, minimizing or even eliminating the risks associated with contamination [38]. The Agar medium has been identified as a reliable culture-medium for

several microbes [39], and it was used to isolate the microorganisms from the aforementioned crude oil contaminated soil [40,41]. The *Mueller hinton* agar was used to isolate the thermophilic microorganisms because, it is a non-selective and non-differential medium; thus, allowing almost all the entrained organisms to grow. The *Mueller hinton* agar used for the bacterial growth was 6.3 g per litre, and it was prepared by mixing it with 166.6 mL distilled water. The mixture was then microwaved at 100°C, and thereafter, the mixture was sterilized by autoclaving it at a pressure of 15 Pa and temperature of 121°C for 15 min; this was so as to ensure that the medium remained sterile. The mixture was then cooled to room temperature and poured into six (6) petrel dishes.

Often, nutrients may be added to the medium, making it rich in protein or sugar. Thus, a nutrient broth was prepared by suspending 3.2 g of *Mueller hinton* agar in 83.3 mL distilled water. The mixture was allowed to settle, and the supernatant nutrient broth was sieved out of the solution. The nutrient broth was then sterilized by autoclaving it at a pressure of 15 Pa and temperature of 121°C for 15 min, and cooled to room temperature. 2 g of the produced sandstone from the reservoir formation was introduced into the broth and incubated for 24 h at 37°C.

2.3. Microbe isolation

Using a sterilized wire loop, the swabs were removed from the tubes and inoculated onto 6 plates marked with an indelible permanent marker; E_{41}, E_{42}, E_{43}, E_{44}, E_{45} and E_{46} using the streaking method (Fig. 1). They were then incubated for 24 h at 37°C [1]. All samples were analyzed using the spread plate technique; the technique is often used for liquid samples containing bacteria so that they are easily counted and isolated. The organisms that exhibited similar characteristics of representative colonies and morphologies on each agar used (that is, indicating discrete colonies from each culture plate), were further sub-cultured to get a pure isolate and later sub-cultured on the nutrient agar by the streaking method.

2.4. Primers for Polymerase Chain Reaction (PCR)

A pair of universal primers (NF and NR) that targets the 16S rRNA gene of most bacteria was used for the PCR adapting protocol as mentioned by Carroll et al. (2000). The forward primer (NF, 5′ GGCGGCAK GCCTAAYACATGCAAGT 3′) and the reverse primer (NR, 5′ GACGACAGCCATGCAS CACCTGT 3′) help to amplify the DNA of several bacteria. Another pair of primer which included the forward primer (P2F) 5′ GCGRCTCTCTGGTCTGTA 3′ and a copy of the reverse primer (NR) from the universal primer pair, was applied in the next PCR which also helped to amplify the DNA of Gram-positive bacteria. The third set of primers include a copy of the forward primer (NF) 5′ GGCGGCAKGCCTAA YACATGCAAGT 3′ from the universal primer paired with (N6R) 5′ GTTCCCGAAGGCACC 3'. This set of primers amplified the DNA of the Gram-negative bacteria. The reaction mixture contained 11.3 μl sterile DDW, 4 μl in 5 times PCR buffer, 2 μl $MgCl_2$, 0.5 μl DNTP, 0.5 μl forward primer, 0.5 μl reverse primer, 0.2 μl TAQ, and a 2 μl DNA template making up a total volume of 20 μl.

The PCR products were purified using the Exo-SAP reagent. The Exo-SAP master mix was prepared by the addition of Exonuclease I (Catalogue No. NEB M0293L) 20 U/ul 50 μl and Shrimp Alkaline Phosphatase (Catalogue No. NEB M0371) of 1 U/ul per 200 μl into a 0.6 ml micro-centrifuge tube. A volume of 10 μl of Amplified PCR product was added to 2.5 μl of the Exo-SAP mixture and incubated at 37°C for 30 min after thorough mixing. The reaction was then truncated by heating the mixture at 95°C for 5 min.

2.5. Bacterial cultivation and DNA extraction

With slight modifications to the medium used, the protocols of Garcia et al. [42] and Klein et al. [43] were adopted in cultivating the bacteria. The samples were inoculated on different plates in order to isolate the bacteria prior being incubated at 37°C for 24 h. After incubation, colonies of bacteria that showed growth patterns in the media, were picked separately with a sterile inoculating loop. A discrete colony of CR11, CR35 and CR 49 in overnight broth cultures were used for the 16S rRNA gene sequencing. Following the manufacturer's instruction, the genomic DNA of isolates CR11, CR35 and CR 49 were extracted using a commercial genomic DNA extraction kit (AidLab, China). Universal bacterial primers (27F: f50 AGAGTTTGATCCTGGCTCAG-30 and 1492R: r50-GGTTACCCTTGTTACGACTT-30) were used to amplify the 16S rRNA gene of the selected carbapenem-resistant ESBL isolates in a simplex polymerase chain reaction (PCR). A brief initial denaturation of the PCR mixture was carried out at 94°C for 5 min,

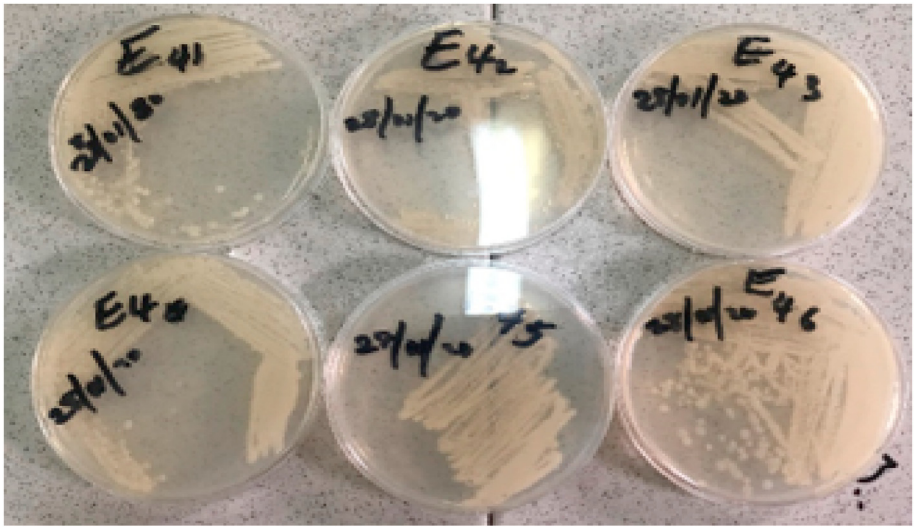

Fig. 1. Microbes isolation process with plates.

followed by 35 cycles at 94°C for 30 s while repeating the cycles at: 52°C for 30 s, 68°C for 60 s and a final extension at 68 °C for 5 min 30 s. The PCR products were analyzed by electrophoresis in a Tris-borate EDTA buffer and viewed using UV fluorescence in a gel image documentation system. The pleural PCR was purified using a PCR clean-up kit (Zymo Research, USA) before being eluted for sequencing [45].

The reference samples were sent to the Inqaba Biotechnical Industries (Pty) Ltd, South Africa for DNA Sequencing. The Blastn algorithms were then used to compare the resulting 16S rRNA gene sequence with the database available from the GeneBank nucleotide (NCBI) [34].

2.6. Thermal capacity tests for the microbes

Two thermotolerant microbes were identified and isolated from a reservoir/formation with temperature above 100°C (212°F). To investigate the thermal capacities of the microbes, Ecotherm Chilling/Heating Dry Bath with digital temperature control was used with synthetic oil as the heating medium. Due to the capacity of the equipment, the microbes were inoculated into tubes and examined at 80–110°C in an oil bath at 1 h interval. The sample recovered at each temperature was inoculated onto a plate and incubated for 24 h at 45°C before analysis.

2.7. MEOR using reservoir permeability tester

Permeability refers to the capacity of a rock-medium to enable movement of fluids under the influence of a pressure differential; this principle is mathematically supported by Darcy's law. To transmit testing fluids into the three accumulators for flooding as shown in Fig. 2, the pump attached to the core-flooding equipment was commissioned in order to transport the microbes from an external container into the stainless steel accumulators having individual capacity of 1.5 L. The reservoir permeability tester operates on three (3) pressure configurations namely; the backpressure, confining pressure, and drive pressure.

The core holders host the reservoir core sample in a sleeve separated by stainless steel spacers which enables a firm connection and serves as a convenient flow connection between the flow line and the core sample. At this region, pressure buildup is activated when the flow is continuous and the signal is sent to the transducer to read the pressure difference between the inlet and outlet in the core chamber. The core sample is placed in the rubber sleeve and the supplied stainless steel spacers were used to fill the gaps on the ends of the core inside the rubber sleeve.

The experiments performed were conducted at room temperature and the temperatures were varied with the help of the flooding equipment (Fig. 3). The core samples used to replicate the oil reservoir was initially saturated with brine, followed by the injection of oil to determine the initial saturation level of the water, S_{wi}. This process ensured the restoration of the core samples to their nominal states by imbibition and drainage displacement. Then, waterflooding was initiated to replicate the secondary recovery process. At this stage, water was pumped into the core plugs until no more oil was recovered in the effluent stream of the core plug. Thus, the residual oil saturation (S_{or}) was obtained. The same process was initiated for the tertiary phase microbial flooding, right from core cleaning to restoration of the core by the imbibition and drainage displacement process. For both the secondary recovery by water flooding and MEOR, the recovery factor was computed by measuring the produced oil in the effluent of the flooding process intermittently.

2.7.1. Properties of the core samples used for the experiment

Six core samples were used for this experimental work. The sandstone core plugs used were obtained from the Niger-Delta region of Nigeria. Each core was first cleaned using Soxhlet extraction method, and its porosity and permeability were measured. Dry weights of the cores were measured, and then immersed in acetone and placed in a vacuum until they became saturated. Acetone helped to dissolve all the mineral oils that came in contact with the cores while drilling them to cylindrical shapes. The weights of the cores were then taken to confirm that the initial weights of the cores were restored before being saturated in deionized water. Finally, the cores were dried until a steady dry weight was recorded for each core. The weight of each core was measured by a measuring scale with an accuracy of ± 0.01 g.

After saturating the core samples, the petro-physical properties of the core samples were determined. The core samples were inserted in the reservoir permeability tester and flooded with brine to determine the absolute permeability of the core samples. By the aid of a pump, brine was injected into the saturated core samples at an initial flow rate of 10 cc/min. The absolute permeability was determined from the readings of differential pressure against time. The porosity and permeability were also determined and tabulated in Table 1. The monophasic permeability test was done, followed by oil injection into the cores until irreducible water saturation was attained. This process created a model reservoir condition in the core plugs.

2.8. Production of biosurfactant

The nutrient broth was prepared in 100 ml of distilled water by dissolving in a measured quantity of the agar. The mixture was allowed to settle down and supernatant solution was decanted. Thereafter, with the aid of an autoclave, it was sterilized at a pressure of 15 Pa and a temperature of 121°C. The nutrient broth was then poured into a sterile universal bottle after the solution was allowed to cool to room temperature. In this medium, 2% bacterial seed culture was inoculated, mixed carefully, and put in a shaker/incubator rotating at 150 rpm at 30°C for 4 days. After incubation, the fermentation broth was centrifuged (12,000 rpm, 20 min) to obtain cell free supernatant solution. Table 2 shows the composition of the mineral salt medium (MSM) used for the production of the biosurfactants.

Bacterial isolates were grown with kerosene and interchangeable carbon source at a ratio of 20:1. 1000 ml of distilled water was applied to it and autoclaved for 15 min at 121°C and 15 psi. The broth was poured into the mineral salt medium and incubated for 72 h at 37°C, 90°C and 110°C. At the end of the fermentation, the cultures were centrifuged at (12000 rpm for 1 h 30 min) to remove the cells. The residue was discarded, meanwhile, the cell-free supernatant was adjusted to a pH 7 using 1 M NaOH and the biosurfactants solution obtained was stored at −7°C.

2.9. Biosurfactant surface tension measurement

The Park et al. [14] method of measuring interfacial tension was adopted in this study. The interfacial tension was measured by the pendant drop method, and the contact angle was measured using the sessile drop method. The inner volume of the cell was first filled with brine so as to culture the microorganisms when measuring the dodecane–brine interfacial tension. The contact angle of the oil–brine system was measured by placing an oil droplet on the disk using the capillary tube.

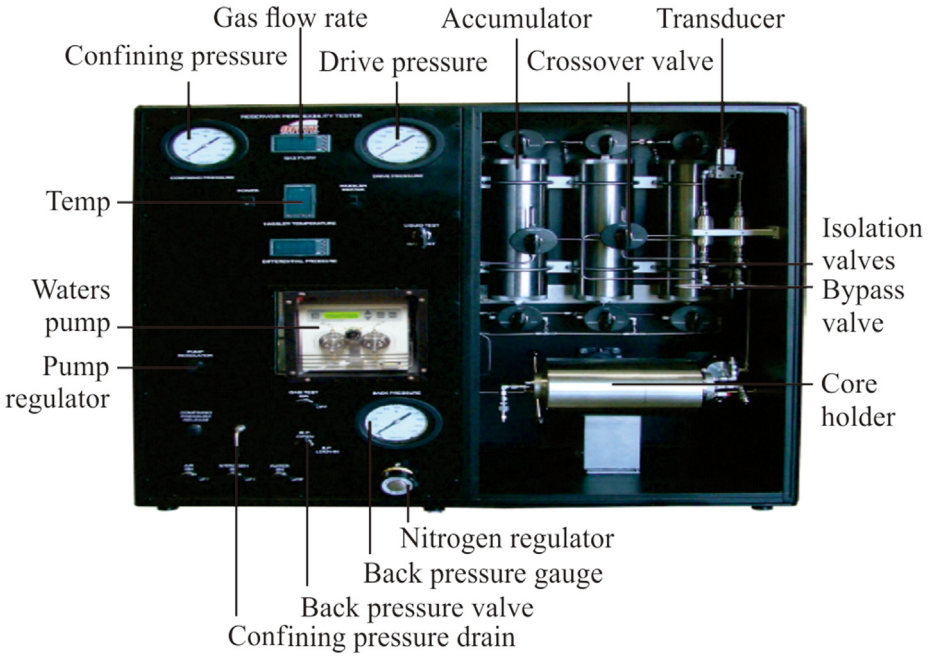

Fig. 2. Reservoir permeability tester (reservoir permeability tester manual).

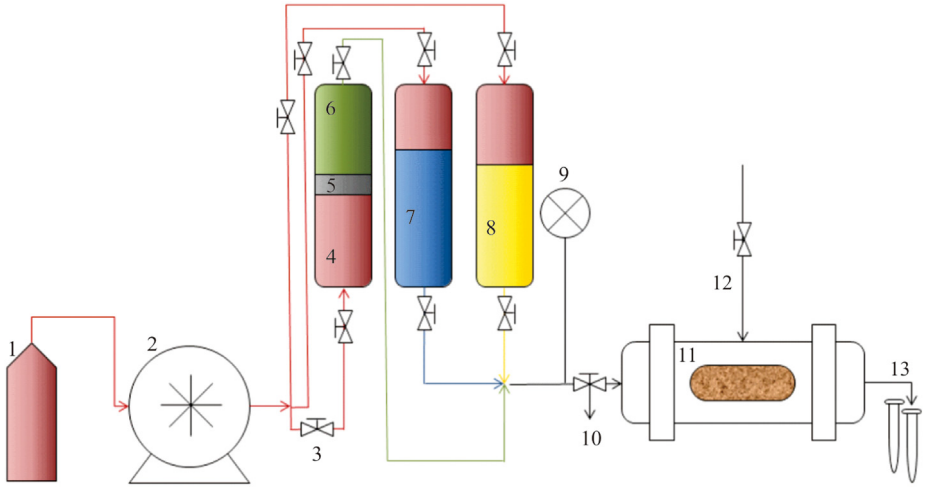

Fig. 3. Core Flooding Setup: 1) pump fluid, 2) pump, 3) valves, 4) displacing reservoir fluid, 5) piston to separate the oils, 6) crude oil, 7) NSB, 8) biosurfactant 9) pressure gauge, 10) bypass valve, 11) Hassler cell holder, 12) valve, and 13) collection container [7].

Table 1
Petrophysical properties of all the core samples.

Sample Name	Length(cm)	Diameter (cm)	Pore volume (cm^3)	Bulk Volumes	Porosity	Permeability (mD)
R1a	4.4 ± 0.1	3.5 ± 0.2	12 ± 0.4	48.11	0.25 ± 0.02	298 ± 2
R1b	4.4 ± 0.1	3.5 ± 0.2	10 ± 0.4	42.34	0.24 ± 0.02	274 ± 2
R2a	4.4 ± 0.1	3.5 ± 0.2	9 ± 0.4	42.34	0.22 ± 0.02	268 ± 2
R2b	4.7 ± 0.1	3.5 ± 0.2	9 ± 0.4	45.23	0.22 ± 0.02	253 ± 2
R3a	4.7 ± 0.1	3.5 ± 0.2	13 ± 0.4	45.23	0.29 ± 0.02	303 ± 2
R3b	4.7 ± 0.1	3.5 ± 0.2	10 ± 0.4	45.23	0.22 ± 0.02	293 ± 2

2.10. Eclipse modification for the MEOR simulation

Simulation studies on microbial enhanced oil recovery options are yet to be extensively captured in literature. The effects of surfactant concentrations and types have been fully studied by Abraham et al. [44], while the options for reservoir simulation to enhance recovery in oil rims was also discussed by Olabode et al. [7]. Its advanced use in conjunction with a gas phase injection to create foam in-situ, creates a barrier between the oil and an ever expanding gas cap. The Eclipse simulator was modified to simulate

Table 2
The Mineral Salt Medium (MSM) and concentration used for biosurfactant production.

Mineral Salt Medium (MSM)	Concentration (g/L)
Monopotassium phosphate (KH_2PO_4)	2.0
Dipotassium phosphate (K_2HPO_4)	5.0
Ammonium sulfate $(NH_4)_2SO_2$	3.0
Sodium chloride ($NaCl$)	0.1
Iron(II) sulfate heptahydrate ($FeSO_4.7H_2O$)	0.01
Magnesium sulfate heptahydrate ($MgSO_4.7H_2O$)	0.2
Calcium chloride dihydrate ($CaCl_2.2H_2O$)	0.01
Manganese sulfate monohydrate ($MnSO_4.H_2O$)	0.002

MEOR because, these options were not available. Two changes were made, the first is the addition of the IFT table based on microbes concentration in order to replace existing data files containing only microbe concentration. Secondly, a volumetric sweep efficiency term in the Eclipse's field oil efficiency equation was also added. Detailed steps of the modification can be seen in the work by Haq et al. [19].

The limitation of surfactant model in Eclipse are: (i) it does not include the detailed chemistry of the surfactant but models only the important features of a surfactant flood on a full field basis and (ii) it does not model the phase behavior of the surfactant.

A total of 5 different surfactant input files were inputed, and these include the IFT with surfactant concentration, viscosity and adsorption. An IFT and biosurfactant concentration table is introduced as an input and used to calculate:

1) Capillary pressure:

$$P_{cow} = P_{cow}(S_w)\frac{ST\left(C_{biosurf}\right)}{ST\left(Cbio_{surf}=0\right)} \tag{1}$$

Where, $P_{cow}(S_w)$ is the capillary pressure which is deduced from the immiscible curves initially scaled to the interpolated end points calculated in the relative permeability model; P_{cow} is the oil-water capillary pressure; $ST(C_{biosurf}=0)$ is the surface tension at zero concentration; $ST(C_{biosurf})$ is the surface tension at the present biosurfactant concentration.

2) Relative permeability: The keyword SURFCAPD is used to define an interpolation parameter F_{kr} as a tabulated function of Log_{10} Nc. The weighting function F can be expressed as:

$$F = F_{kr}(log_{10}N_c) \tag{2}$$

3) Capillary number, N_c:

$$N_c = \frac{|K \cdot grad\ P|}{ST}C_{unit} \tag{3}$$

$$|K \cdot grad P| = \sqrt{(K_x \cdot grad P_x)^2 + (K_y \cdot grad P_y)^2 + (K_z \cdot grad P_z)^2} \tag{4}$$

Where K is the permeability, ST is the interfacial tension; P is the potential; and C_{unit} is the conversion factor depending on the units used.

For a particular cell i,

$$K_x \cdot grad P_x = 0.5\left[\left(\frac{K_x}{D_x}\right)_{i-1,j} \cdot (P_i - P_{i-1}) + \left(\frac{K_x}{D_x}\right)_{i,j+1} \cdot (P_{i+1} - P_i)\right] \tag{5}$$

Eqn. (5) can be generated similarly for y and z directions.

4) Water viscosity: the water-biosurfactant solution viscosity was calculated using equations (6) and (7):

$$\mu_{ws}\left(C_{biosurf},\ P\right) = \mu_w(P)\frac{\mu_{biosurf}\left(C_{biosurf}\right)}{\mu_w\left(P_{ref}\right)} \tag{6}$$

For the Brine, equation (6) can be expressed as a function of salt concentration as well:

$$\mu_{ws}\left(C_{biosurf},\ P,\ C_{salt}\right) = \mu_w(P, C_{salt})\frac{\mu_{biosurf}\left(C_{biosurf}\right)}{\mu_w\left(C_{salt-ref},\ P_{ref}\right)} \tag{7}$$

5) Biosurfactant adsorption: the quantity of the biosurfactant adsorbed on the rock is given by:

$$M_{AS} = PV_{cell} \cdot \frac{1-\varnothing}{\varnothing} \cdot \rho_{mas} \cdot CA\left(C_{biosurf}\right) \tag{8}$$

Where ρ_{mas} is the mass density of the formation rock; $CA(C_{biosurf})$ is the adsorption isotherm as a function of the biosurfactant concentration in solution; M_{AS} is the mass of adsorbed biosurfactant; $\varnothing$ is the porosity of the formation rock; and PV_{cell} is the pore volume of the cell.

This study used constant viscosities and adsorption values to investigate the effect of IFT on different concentrations of surfactants and alcohol. Since the Eclipse surfactant model does not take into account the chemical properties and phase behavior of the surfactant, the introduction of detergent concentrations and surfactant solution and IFT tables may give acceptable results. In order to achieve good oil recovery, it is recommended to add detergent losses when designing the detergent filling system and this should be done in such a way that the detergent concentration exceeds the CMC.

In order to be more accurate, these variables are made heterogeneous in nature, thus, making each cell in the model have its own individual properties which are different from those of the next cell. For hydrocarbon fluids to flow through the reservoir to the point of least resistance due to drawdown (producer well), the cells must be connected via properties such as porosity and permeability (all which have been included within the heterogeneous nature of the model). If the porosity values of some cells are made zero, then such cells are inactive with no reservoir properties. The size of each cell in the x, y and z directions has been described in the grid section of the model as DX, DY and DZ and these sizes can be varied depending on the nature of the project. A reasonable size was adopted for each cell in specific directions to fully estimate the mathematical fluid in place (FIP) volumes of the model. The interaction of the produced biosurfactants was estimated using data estimated from the experiments in conjunction with biosurfactant keywords such as SURVICS (which is the biosurfactant viscosity) and SURFEST (the surface tension property).

To execute a microbial flooding option using a reservoir simulator (Eclipse), a 5 × 5 × 1 simple model was created. The model had input variables of oil, water and microbes, within lab unit

options. The grid property options are as shown in Table 3. The fluid densities for oil and water are 850 g/cc and 1000 g/cc respectively. The simulation was initiated at a reference pressure of 270 atm, while the water formation volume factor, water compressibility, rock compressibility and oil viscosity were 1.03 rcc/sec, 4.6e^{-5}/atm, 3.0e^{-6}/atm and 0.34 cp respectively. Table 4 shows the PVT property of the dead oil with no dissolved gas.

The surfactant solution viscosity functions (SURFVISC) and water/oil surface tension versus the concentrations (SURFST) for microbes A and B are shown in Table 5. The saturation functions for the reservoir oil and water are shown in Fig. 4(a) and (b) respectively.

The rate of microbe adsorption on the rock surfaces were calculated via a keyword (SURFADS). The adsorption function of each microbe is shown in Table 5. The rock had an adsorption index of 1 and a mass density of 2650 g/cc. The keyword (SURFCAPD) is used to describe the microbe capillary desaturation functions (Table 6). The de-saturation function describes the transition between immiscible conditions (low microbe concentration) and miscibility (high microbe concentration) as a function of the dimensionless capillary number. The keyword EQUIL is used to input a datum depth of 2600 cm, pressure at datum depth of 270 atm and water oil contact of 2700 cm. Fig. 5 shows the model's initial oil saturation for the producer and injector wells.

Two wells (OP and INJ) were initiated as oil producer and water injector wells respectively. The well completion dimensions using COMPDAT are as shown in Table 7. The producer and injector wells were set under reservoir control. The keyword, WSURFACT is used to describe microbe concentrations for the injector wells.

Four case studies were considered. The first 3 case studies are essential for estimating oil recovery/production before the commencement of microbial flooding.

1. Oil production total from primary recovery
2. Oil recovery from water injection
3. Oil recovery from microbe A injection
4. Oil recovery from microbe B injection

The crude oil used for in study was characterized using ASTM Test Methods and the properties are tabulated in Appendix B (Table B1).

3. Results and discussion

3.1. Isolation and DNA purity of the microorganisms

A total of two (single & double) microbial colonies were picked up from the samples. They were sub-cultured into new plates to optimize the microbial colony growths. Pure cultures of two isolates (Raised, R and Flat, F) were prepared by streaking samples of the enrichments on the plates containing the colonies. All isolates were then inoculated on slants and preserved at 4°C.

The Spectrophotomeric method which measures the absorbance of the sample was adopted for nucleic acid quantification and DNA purity (260/280 ratio of the absorbent). In order to accurately measure the concentration of a substance based on its absorbance, it is necessary to identify the wavelength of light that the substance maximally absorbs. For Diribonucleic acid (DNA), the maximum absorbance is at a wavelength of 260 nm. But proteins which act as non-nucleic acid contaminants maximally absorb UV light at 280 nm, whereas, the ratio of nucleic acid to protein (A260/A280) is generally used as an indicator of the purity of DNA samples. The purity levels for the DNA extract-isolates R and F are 1.75 and 1.76, respectively, thus indicating very high purity levels for the bacteria isolated from the sandstone reservoir (Table 8). Literature has shown that pure DNA has DNA purity ratio A260/A280 which is approximately 1.8; this means that there was negligible or no significant contamination of the DNAs obtained from both bacterial strains [46,47].

Table 4
Oil properties.

Pressure (atm)	FVF (rcc/scc)	Viscosity (cp)
200	1	0.47
280	0.999	0.47
300	0.999	0.47

3.2. Identification of the isolated petroleum microbes

The BLASTn search of the nucleotide sequence with the most similar 16S Rrna gene sequences of the GenBank database (http://www.ncbi.nlm.nih.gov/blast) revealed the closest sequence that conformed to the genes in the sequence-database. The results revealed that the bacterial strain R had some semblance with *Bacillus nealsonii* with 98.02% similarity, while bacteria F exhibited similar characteristics with *Bacillus amyloliquefaciens* with about 98.93% similarity. The corresponding sequences have been documented with the GenBank under accession numbers SUB7530557 13 MT542325 and SUB750557 50 MT542326.

3.2.1. Produced biosurfactant characterization

FTIR analyses of the produced biosurfactants showed the presence of carboxylic functional groups and aliphatic amines that represent peptide bonds of lipopeptide biosurfactants. *Bacillus nealsonii* showed various absorbance bands, characterized by aliphatic amines at 1023 cm^{-1}- 972 cm^{-1} (Fig. 6a) and those of *Bacillus amyloloquefaciens* (Fig. 6b) respectively resulting in stretching vibrations of C–N bonds. Moreover, band formation at 1045.92 and 862.03 cm^{-1} are associated with stretching vibrations that are observed for glycosidic linkages. The 1250–1020 cm^{-1} peak, indicates the presence of C–N stretching aliphatic amines. The peaks observed at 1453.40 and 1124.36 cm^{-1} suggest stretching bands between carbon atoms and hydroxyl groups. The C=O stretch mode of 1762 cm^{-1} and 1757 cm^{-1} as seen for both biosurfactants ranging from 1690 to 1762 cm^{-1}1 correspond to ester carbonyl groups characterized by peptides. Another peak ranging from 3500 to 3200 cm^{-1} gave an indication of alcohols and O–H stretch phenols and H–bonds present. The above results have also been reported in literature that the presence of peptides and aliphatic hydrocarbons are indicative of the lipopeptide class of biosurfactants.

Table 3
Grid property options.

XX permeability (mD)	YY permeability (mD)	ZZ Permeability (mD)	Porosity	TOPS (cm)	X grid block sizes (cm)	Y grid block sizes (cm)	Z grid block sizes (cm)
4500	4500	1050	0.25	2600	50	50	0.58

*XX, YY and ZZ connote a three dimensional (3D) grid system.

Table 5
Microbe solution viscosity function.

SURFVISC (A)		SURFVISC (B)	
Concentration (g/cc)	Viscosity (cp)	Concentration (g/cc)	Viscosity (cp)
54.0	1.65	54.0	1.98
Concentration (g/cc)	ST_{wo} (dyne/cm)	Concentration (g/cc)	ST_{wo} (dyne/cm)
54.0	18.8	54.0	12.5
Ssl (g/cc)	Ssc (g/g)	Ssl (g/cc)	Ssc (g/g)
0	0	0	0
54	12	58	19

*Ssl: is the local microbes concentration around the rock.

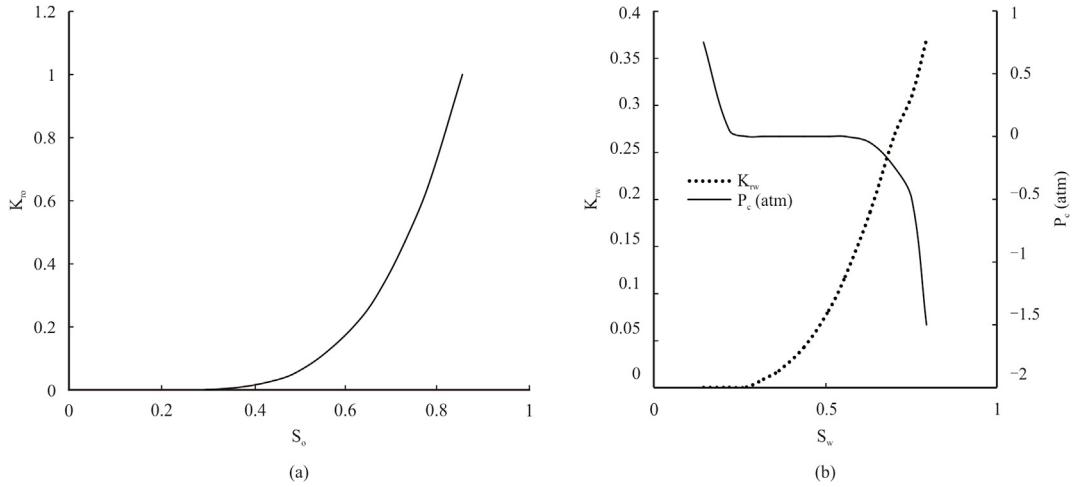

Fig. 4. (a)Oil saturation function. (b)Water saturation function

Table 6
Surfactant capillary desaturation functions.

Log(CAPN)	Fm
−8	0
−3.5	0
−1	1
−7	1

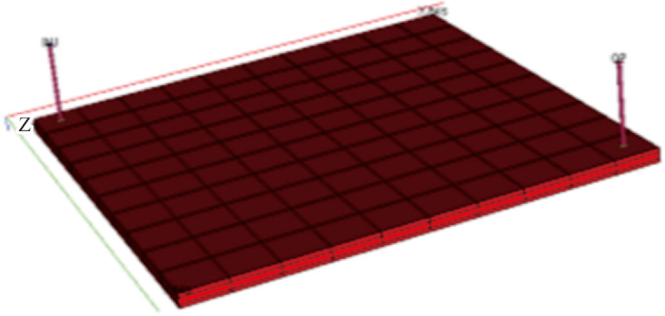

Fig. 5. Initial oil saturation.

Table 8
DNA concentration and purity of isolates.

Isolation code	DNA concentration	DNA purity
R	86.6	1.75
F	50.5	1.76

3.3. Thermal capacity of the petroleum microbes

The pour plate method was adopted in counting the number of colony-forming bacteria present in the nutrient agar medium after each temperature test. Fig. 7 shows the colonies that grew within and on the solid medium; each colony was carefully counted. The results show that the growth of *Bacillus amyloliquefaciens* became scanty after 100°C and no significant growth was recorded after 110°C. *Bacillus nealsonii* showed mild growth after 110°C which is the maximum temperature for the Ecotherm Chilling/Heating Dry Bath used for heating the isolated microbes. The plate count method or spread plate relies on the bacteria growing into a colony on a nutrient medium. The colony then becomes visible to the naked eye such that the number of colonies on a plate can be counted. *Bacillus amyloliquefaciens* yielded moderate growth of

Table 7
Well completion dimensions.

Well OP				Well INJ			
i	j	K (upper)	K (lower)	i	j	K (upper)	K (lower)
10	10	1	3	1	1	1	3

*i, j and k are the coordinates

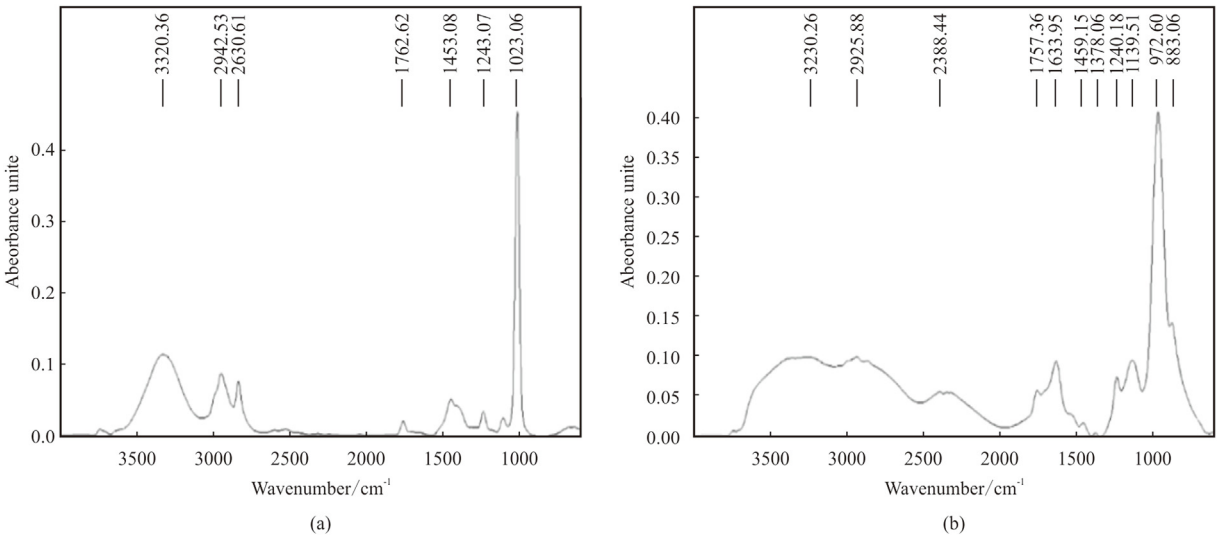

Fig. 6. FT-IR analysis for (a) *Bacillus nealsonii* and (b) *Bacillus amyloloquefaciens*.

bacterial colonies after 24 h of incubation at 80–90°C, with mild to scanty bacterial growth at 100–110°C whereas, *Bacillus nealsonii* yielded significant to moderate growth at 80–100°C, and mild growth of bacteria colonies at 110°C (Fig. 7). Thus, *Bacillus nealsonii* demonstrated higher thermal tolerance/resistance relative to *Bacillus amyloliquefaciens.*

3.4. MEOR core flooding analysis at temperature of 27°C

The results were generated from experimental core flooding processes using reservoir core samples of similar petro-physical properties with a known reservoir. The produced *Bacillus nealsonii* and *Bacillus amyloliquefaciens* were used in carrying out tertiary enhanced oil recovery. The performance of these microbes were analyzed based on their behaviors and influences when in contact with injected crude oil within the rock formation. The flooding process started after oil was injected into the core to displace any brine present. This was done to replace the brine saturated core with crude oil as well as determine the original oil in place (OOIP).

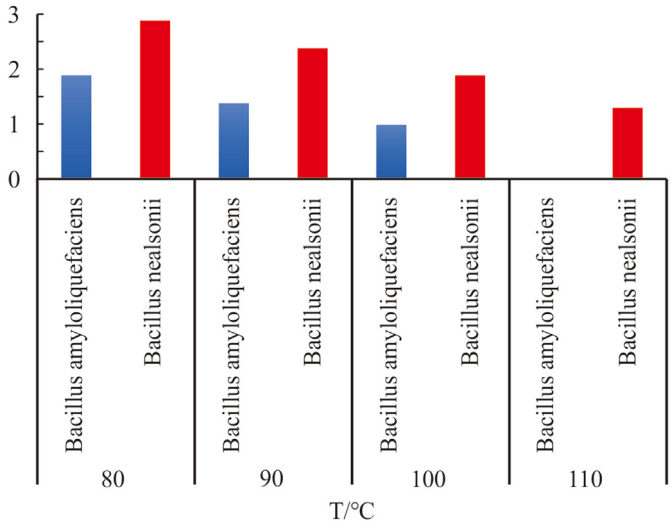

Fig. 7. Identification of microbes thermal capacity using total bacteria count with nutrient agar.

The results of the recovered crude oil after water flooding (secondary recovery process) are presented in Fig. 8.

Bacillus amyloliquefaciens was tagged microbe A, while *Bacillus nealsonii* was tagged microbe B. However, flooding experiments with *Bacillus amyloliquefaciens* commenced sequel to water flooding on the core samples. *Bacillus amyloliquefaciens* (microbe A) was injected as the test-fluid and flooded through core sample R1a. A similar flooding procedure was employed in flooding core sample R1b. Flooding with *Bacillus nealsonii* (microbe B) also commenced after water flooding on core sample R1b. The flooding experiment was done at a confining pressure of 3.0 MPa. Fig. 9 shows the recovery factor for the two microbes with different pore volumes injected at a fixed injection rate of 0.5 cc/min. The result from Fig. 9 shows that a maximum of four (4) pore volumes were injected and that the microbe B gave higher recovery based on its recovery potential at temperature of 27°C.

Core samples R2a and R2b were used for the same flooding process with microbes A and B respectively. The estimated recovery factors for microbes A and B at a confining pressure of 3.0 MPa and temperature of 27°C were 46.4% and 48.6% respectively; which is higher than that (26.9%) obtained for water flooding. The recovery factor of microbe B is higher compared to that of microbe A (Fig. 9). When core samples R3a and R3b were flooded with microbes A and B respectively, the same trend was observed after the tertiary enhanced oil recovery process. That is, microbe B gave a maximum oil recovery factor of 48.3% compared to the value (41.7%) obtained for microbe A. Based on the result in Fig. 10, the microbes gave similar performance when the pore volume was between 0.1 and 2 cc, however, when the pore volume was between 2.5 and 3.1 cc and above 2.5, sample core R2a gave a higher recovery factor but peaked at 3.1 owing to the maximization of oil-recoverability stimulated by maximum growth and very near death-phase of the microbes; this in turn justifies the reason for the increased trend seen for the Core R2b samples which maximized oil recovery owing to the little or no competition exhibited by its counterpart.

Reports from thorough experimental investigations on the synergy between the use of controlled salinity waterflooding (secondary recovery) and biosurfactants injection for EOR at relevant reservoir conditions of temperature were also conducted. Fig. 11 shows the volume of crude oil produced with time, and the trend showed that water (brine) available during secondary recovery could no longer produce crude oil at 95th minute, thus some

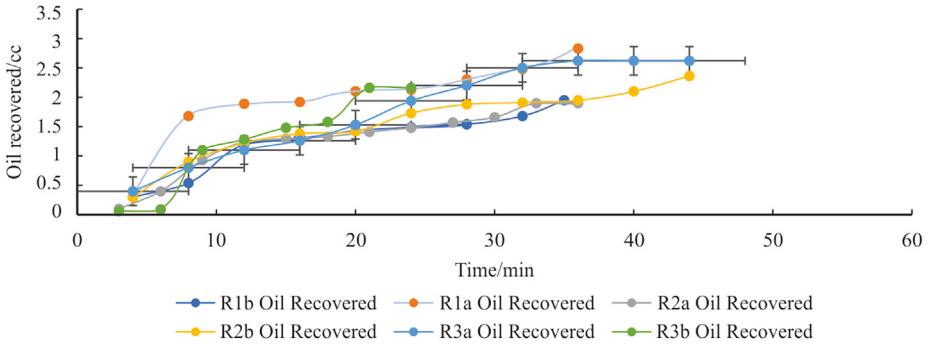

Fig. 8. Volume of Oil Recovered during Water Flooding at a flowrate of 0.5 cc/min.

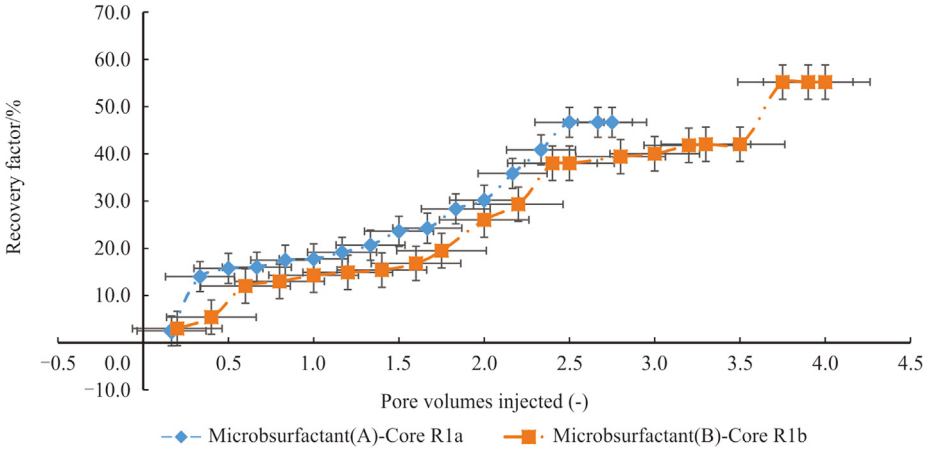

Fig. 9. Comparison of Recovery Factor for the Two Microbes on R1a and R1b cores.

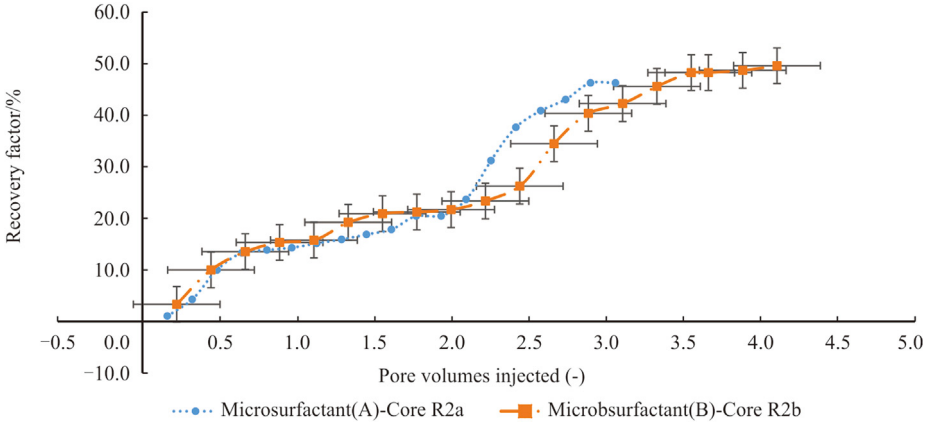

Fig. 10. Comparison of Recovery Factor for the Two Microbes on R2a and R2b cores.

of the crude oil were left in the pore spaces. The trend shows that despite the recovery factor from secondary recovery process, significant additional volumes of crude oil are expected to be recoverable through tertiary recovery or enhanced oil recovery methods. The biosurfactant application as tertiary recovery and enhanced recovery method led to a further recovery of about 68.42%. The incremental oil recovery using bio-surfactants as enhanced recovery method after secondary recovery was significant. Mobilization of the entrapped crude oil required either an increase in viscous forces and/or a reduction of capillary forces in the reservoir. Biosurfactants reduce oil-water interfacial tension, which provides greater increase in capillary number needed for substantial oil recovery. The impact of enhanced recovery on crude oil production can be significant given that an increase in the recovery of crude oil by 1% merely translates to an impressive yield of 70 billion barrels of global oil reserves. The biosurfactant from the microbes was able to extract up to 68% of the residual oil left in the core sample after primary and secondary recovery processes. Current literature shows that oil movement through porous media such as hydrocarbon reservoirs is facilitated by altering the interfacial properties

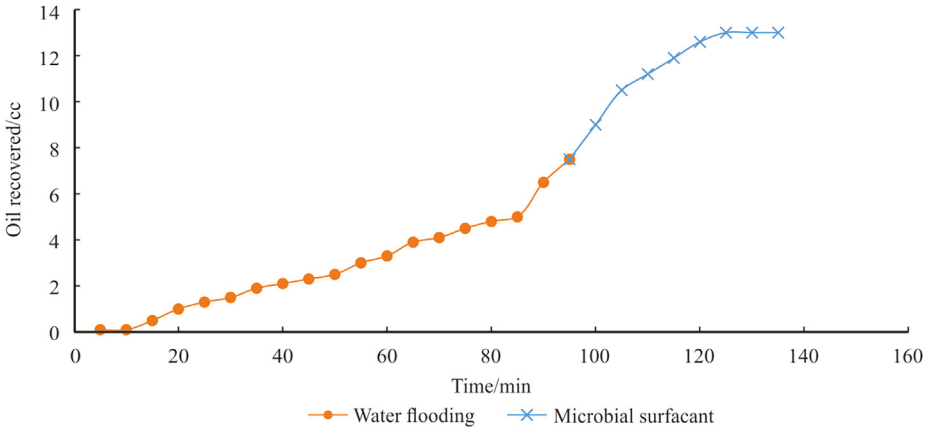

Fig. 11. Secondary and Tertiary Incremental Oil recovery against time.

of the oil-water minerals – displacement efficiency (decrease in the IFT results in an increase in permeability), driving force (reservoir pressure), fluidity (miscible flooding; viscosity reduction), and sweep efficiency (selective plugging; mobility control) [35]. When poor oil recovery from oil wells is due to low permeability of the rock formation, or due to the high viscosity of the crude oil, the ability of biosurfactants to reduce IFT between the flowing aqueous phase and the residual oil saturation can potentially improve the process efficiency and recover more oil. Biosurfactants can also potentially reduce the capillary forces that prevent oil from moving through rock pores.

3.4.1. MEOR core flooding analysis at elevated temperature

According to Niu et al. [48], the overall MEOR method, depends on parameters such as depth, the viscosity of oil, permeability, pH, temperature, pressure, water reduction, the specific gravity of crude oil, brine salinity, porosity, residual oil saturation, wax content, and the microbial species involved. The temperatures adopted in this study are 80, 90, 100, 110 and 120°C as presented in Refs. [48,49]. Core samples R1a and R1b were used in carring out MEOR at different temperatures. Fig. 12 shows the temperature effect on microbes A and B used in tertiary enhanced oil recovery for cores R1a and R1b respectively. It was observed that at all temperatures, microbe B gave higher range of oil recovery factor (55.2–64.1%) compared to microbe A whose value is in the range of 46.7–57.5%. There was an initial increment in oil recovery factor as the temperature increased from 80 to 110°C, but, this increment in recovery factor was constant between 110 and 120°C. This trend can be attributed to the thermal tolerant test that showed a scanty growth of the microbes above 110°C.

Fig. 13 shows the incremental percentage recovery at increased temperatures. There were incremental recoveries between 80 and 110°C, but, a constant trend was observed with respect to each sample at 110–120°C.

The same initial increment in oil recovery factor was observed for core samples R2a and R2b as the temperature increased from 80 to 110°C, and was constant for each sample between 110 and 120°C when microbes A and B were used for the MEOR process (Fig. 14). Fig. 15 also shows an incremental recovery between 80 and 110°C, but, a constant trend between 110 and 120°C.

After conventional oil recovery operations (primary and secondary recovery processes), abundant residual oils may still be trapped in the pore spaces of complex capillary networks in the reservoir [48]. Hence, due to insufficient extraction, the average recovery rate is reduced to 30%. As a promising tertiary oil recovery technique, microbial enhanced oil recovery (MEOR) which involves the use of indigenous screened microorganisms as applied and reported in this study, were used to enhance crude oil recovery in the reservoir core samples. The results show that temperature has effect on the microbial recovery factor and this trend was also observed in the mathematical modelling and numerical simulation of the effect of temperature on EOR by Chakraborty et al. [50]. Kögler et al. [51] also observed a high incremental oil recovery for sandpacks (core plugs), and attributed the success of the MEOR mechanism to wettability alteration, matrix dissolution and bioplugging. In comparison to primary and secondary recovery methods, MEOR is undeniably a better alternative, as its contributions to crude oil recovery entails a more economically feasible process. The observed trends in the recovery factor and incremental recovery showed that the two thermotolerant petroleum microbes are responsible for the enhanced oil recovery recorded from the MEOR process. Saravanan et al. [52] highlighted that microorganisms enhance oil recovery by producing various metabolites, thus, MEOR is an effective alternative tertiary method for oil recovery among other enhanced oil recovery (EOR) methods. Alvarez et al. [53] after using *Bacillus subtilis* to produce a surfactin for EOR, also observed a high oil recovery and they concluded that the surfactin is an excellent candidate for MEOR subsurface application. This study adopted the in-situ technique where the produced thermotolerant petroleum microbes were injected into the reservoir core samples for tertiary enhanced oil recovery. Gao [54] reported good success rates of MEOR from field experiences in China. The report also highlighted reservoir temperature as one of the obstacles for massive field application, but the study suggests that careful selection of microbes has been proven very effective for application under challenging reservoir conditions [55]; this was also observed in this study where the thermotolerant petroleum microbe (*Bacillus nealsonii*) gave a high recovery factor (64.1%) at 110°C.

According to Ke et al. [21], significant or insignificant variation in porosity and permeability properties may be the key factors responsible for enhanced oil recovery. Thus, in this study, the nonvariation in these properties, suggests that the microbial activities of both microbes are the key factors responsible for the recorded MEOR results. Laboratory-based core column flooding evaluation in literature, have shown that synthetic indigenous microbial strain increased oil recovery and effectively reduced the interfacial tension [21]. MEOR has various mechanisms acting in combination for improved oil sweep and microscopic displacement efficiencies [27]. Fig. 16 shows the interfacial tension (IFT) test-results obtained in this study. The reduction in IFT contributes to wettability alteration and oil-detachment from the formation pore surface which in turn influence hydrocarbon recovery. Similar

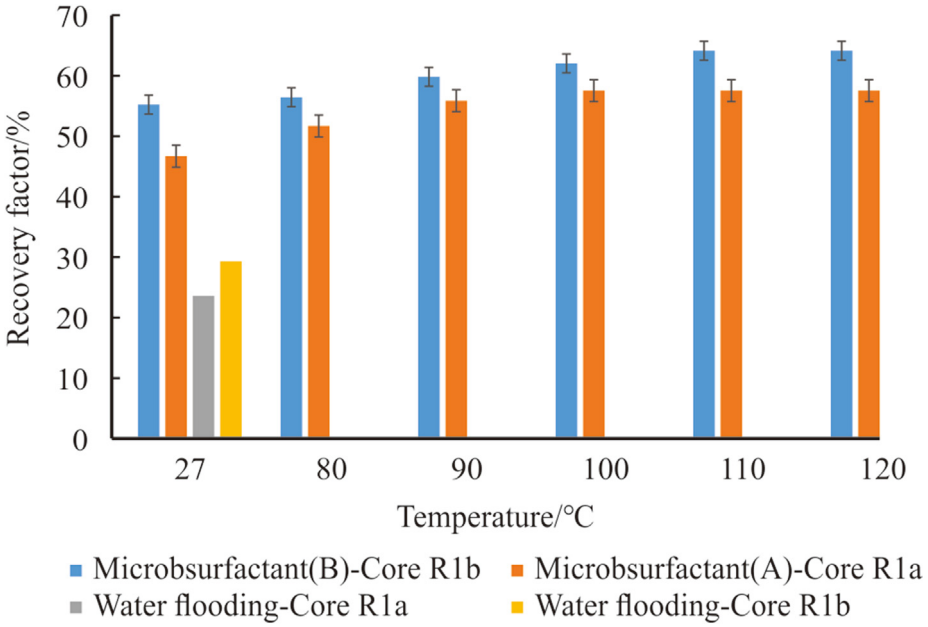

Fig. 12. Temperature effect on microbes A and B recovery factor.

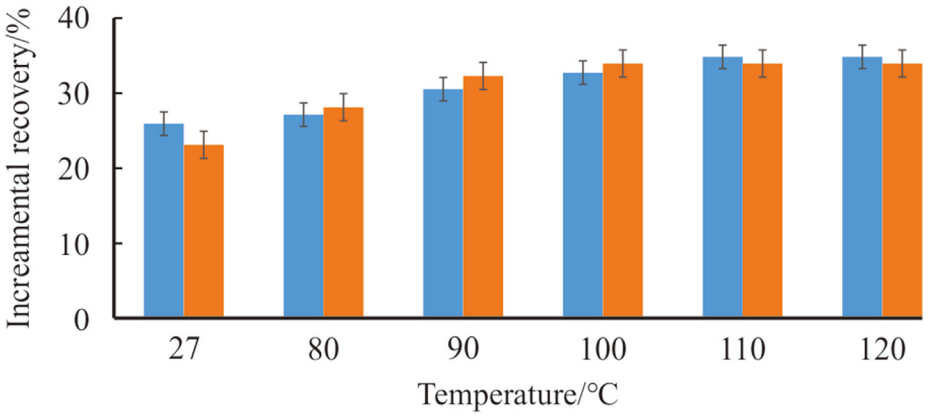

Fig. 13. Incremental recovery of microbes A and B at different temperatures.

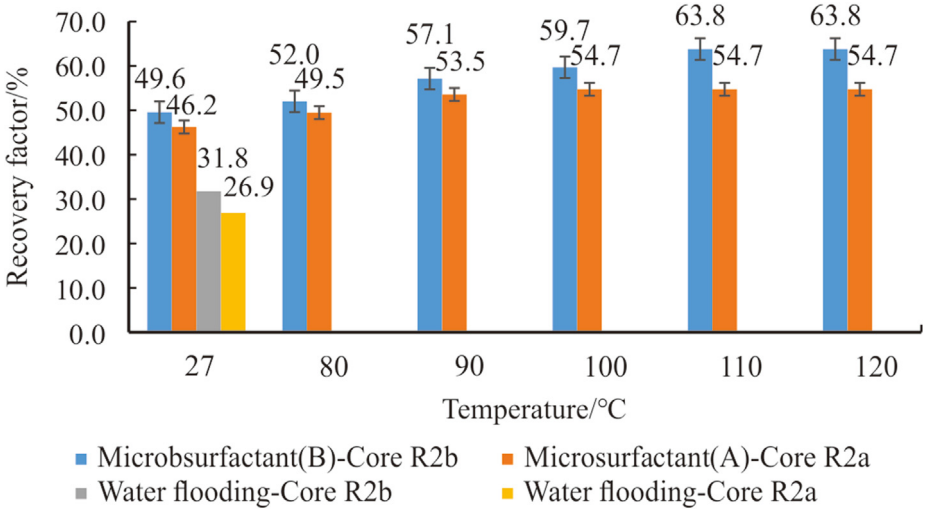

Fig. 14. Temperature effect comparison for Microbes A and B.

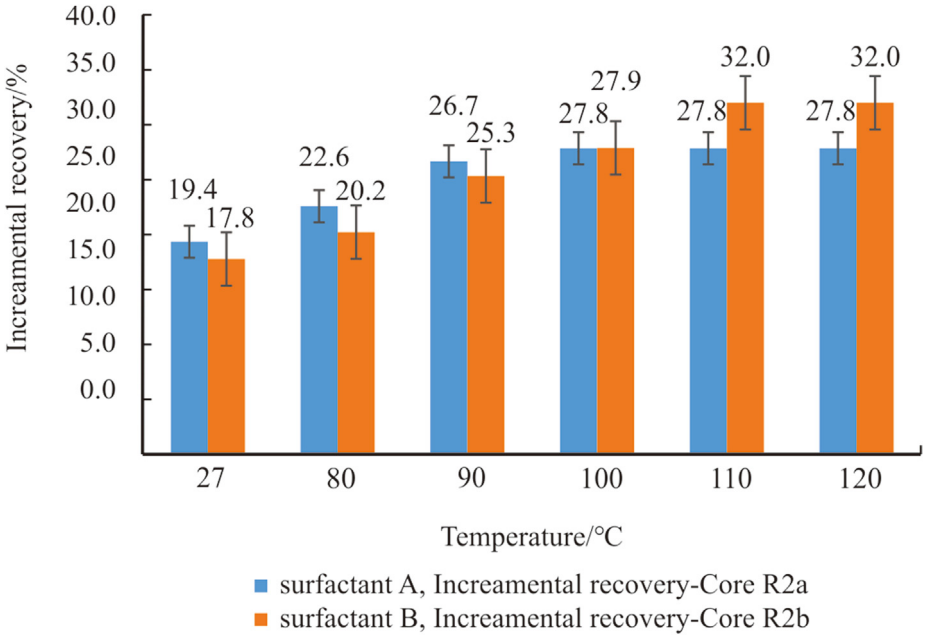

Fig. 15. Incremental recoveries comparison for microbes A and B.

trends in the alteration of microbes IFT have been recorded by Putra and Hakiki [56]. Wettability of the porous media is one of the most important parameters influencing distribution, saturation, and hydrocarbon flow, thus directly affecting the hydrocarbon recovery [57]. The results in Fig. 16 show that the petroleum microbe B decreased the tension at the oil-water interface more than that obtained for microbe A at the three temperatures under consideration. This decrease in IFT helped to detach oil from the formation pore surfaces, since the bulk displacement of hydrocarbon is possible if the capillary forces that entrap the crude oil are reduced. Literature has highlighted that the IFT between the hydrocarbon and water phases is largely responsible for crude oil trapping in the formation matrix [58]. The reduction rate of IFT increased as the temperature increased. The reduction in IFT promotes the formation of emulsion which often resists the flow from high permeability sections, thus forcing flow into the low permeability regions.

As biosurfactant migrates towards the oil-aqueous interface, the IFT is reduced while capillary number increases, thus displacing and recovering residual oil. Biosurfactants ease IFT and surface tension, in addition to enabling O/W or W/O emulsion stabilization, which in turn increases the mobility of insoluble organic compounds. They further alter the wettability of the well-bore rocks to

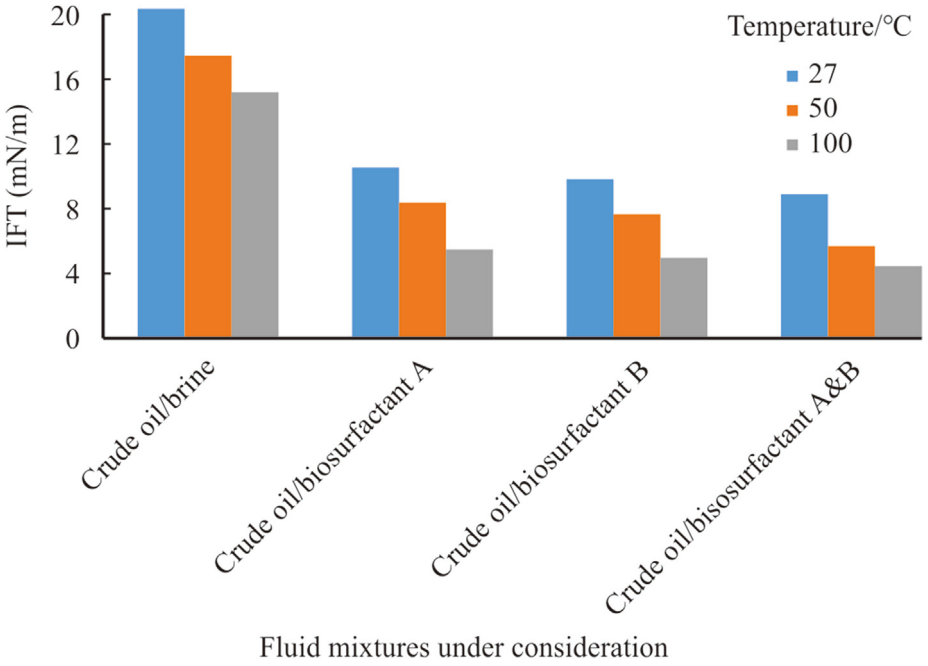

Fig. 16. Interfacial Tension response of the Fluid Mixtures.

Table 9
Water injection and microbe B injection.

	Simulated production (cc)	Recovery factor (%)
Water flooding	65,180	64.48
Microbe B	70,761	70.00

displace more oil flms from the rock pores. Biosurfactants reduce interfacial activity and improve oil recovery proficiency compared to other by-products, hence, they have gained prominence in MEOR processes.

3.5. Core-flooding experiment simulation

3.5.1. Case scenario: primary recovery

This case scenario is the base case of no injection. The oil produced is as low as 500 cc due to a rapid drop in reservoir pressure. Fig. 16 shows the production profile, while Figure A1 in Appendix A (supplemental file) shows the final oil saturation which is not different from the initial oil saturation; this is an indication of low oil production.

3.5.2. Case scenario 2: water flooding

Water flooding is initiated through the injector well at a rate of 1000 cc/hr. The cumulative oil production at this rate is 65180 cc with 64.48% recovery factor as shown in Table 9. The production profile in Fig. 17 describes a static bottom hole pressure of 260 atm. The final oil saturation in Figure A2 (Appendix A; supplemental file), shows a vivid oil depletion compared to the initial value.

3.5.3. Case scenario 3: Microbe B injection

The injection of microbe B at a concentration of 58 g/cc resulted in a total oil production of 70761 cc with a recovery factor of 70% (Table 9). This is the highest oil recovery with a static bottom hole pressure of 270 atm (Fig. 17). The final oil saturation (Figure A3 in Appendix A- supplemental file) shows a visible oil saturation depletion away from the injector well as the color band spreads and moves towards the region of low oil saturation.

3.5.4. Case scenario 4: Microbe a injection

The final oil saturation after injecting microbe B at a rate of 54 g/cc is shown in Figure A4 (Appendix A). It portrays a near semblance with the final oil saturation from water flooding. The production profile is shown in Fig. 17 with a cumulative oil production of 68,356 cc. Table 10 shows the breakdown of the cumulative oil production with about 67.62% recovery factor.

The cumulative oil production from the simulation closely matched that gathered from experimental data, and the slight difference can be attributed to the adjustment of the simulation input parameters. From the simulated oil recovery curve, microbe B gave the highest oil recovery in relation to microbe A, which is a good match with the experimental results. A similar trend was observed in the total oil recovery by Haq et al. [19], when the laboratory and simulated results obtained for the JF-2 microbe were considered for the recovery of butanol. Based on the findings of this study, the recovery factors of both microbes are similar to the results from experiment, although a higher recovery factor was obtained for microbe B compared to microbe A.

4. Conclusions

Previous studies have shown that abundant residual oil is trapped in the pore matrix of reservoir formation after primary oil recovery operations. As a promising method/tertiary oil recovery technique, the MEOR adopted in this work offers the following:

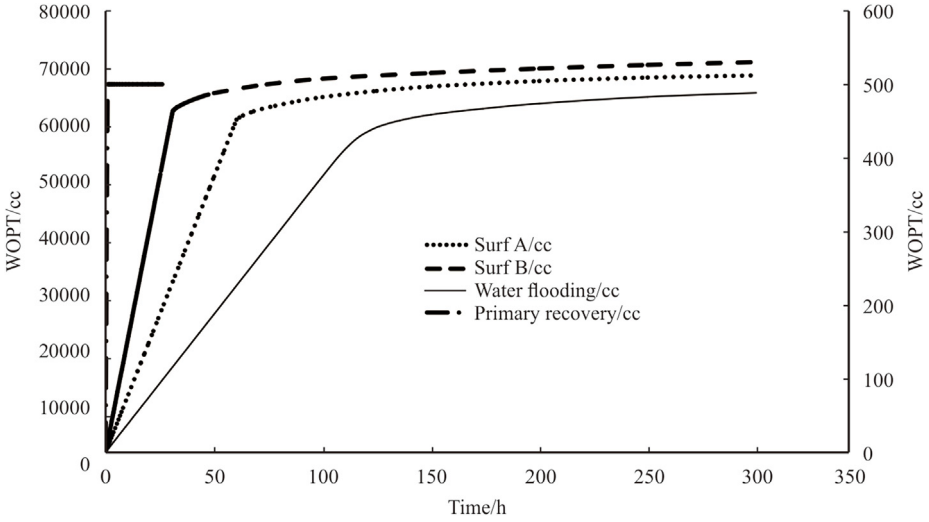

Fig. 17. Summary plots of oil produced for all case scenarios.

Table 10
Cumulative Oil production.

Case scenario	Simulated well oil production total (cc)	Experimental well oil production total (cc)	Recovery factor (%)
Primary recovery	500	–	–
Water flooding	65,180	64, 894	64.48
Microbe A	68,356	68,056	67.62
Microbe B	70,761	70,298	70.00

i. The use of selected microorganisms for the production of specific metabolites by injecting microbes to improve oil recovery which was validated in this study.
ii. The successful isolation and characterization of two petroleum thermotolerant microbes (*Bacillus amyloliquefaciens* and *Bacillus nealsonii*) from reservoir sandstones, were done through culture-dependent method gene sequence analysis.
iii. The thermotolerant microbes were used in carrying out microbial enhanced oil recovery of crude oil. The core-flooding experiments conducted with these microbes were at different temperatures (27–120°C).
iv. The MEOR experimental and simulated results show that both thermotolerant petroleum microbes effectively increased oil recovery at different temperatures with microbe B outperforming microbe A at 110°C.
v. It can be inferred that *Bacillus nealsonii* is a more thermally resistant bacterial strain compared to *Bacillus amyloliquefaciens* at 110°C.
vi. Microbe B decreased the tension at the oil-water interface compared to what was observed for microbe A at different temperatures. The decrease in IFT caused the oil to easily detach from the formation pore surfaces, and the bulk displacement of hydrocarbons was then initiated by the reduced capillary forces that entrapped the crude oil.
vii. It can be concluded that the extracted microbes and their biosurfactants can be considered as low-cost, low-risk potential aids for tertiary/enhanced oil recovery.

Credit author statement

Emmanuel E. Okoro and Ewarezi A. Efajemue: Conceptualization, Methodology, Formal analysis, Original draft preparation, Supervision Oluwasanmi A. Olabode, Temiloluwa Ojo and Oyinkepreye D. Orodu: Data curation, Investigation, Project administration Sanni E. Samuel, Ewarezi A. Efajemue and Emmanuel E. Okoro: Methodology, Writing - review & editing, Formal analysis Emmanuel E. Okoro and Ewarezi A. Efajemue P.: Software, Methodology, Validation, Visualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.petlm.2022.01.008.

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