Microbial Enhanced Oil Recovery using Biosurfactant produced with Hyperthermophiles isolated from Subsurface Sandstone Reservoir

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Abstract.

Microbial enhanced oil recovery (MEOR) among other enhanced oil recovery (EOR) innovation is an important alternative approach for oil recovery when primary and secondary methods aren't productive. The responsible factor for MEOR procedure is the microbes and their by-products like biosurfactants, biopolymers and so on, biosurfactants assume key jobs such as degradation of hydrocarbon pollutants in soil, removal of metals from soil surfaces, dispersion of inorganic minerals in mining and manufacturing processes and so on. This study is focused on the application of isolated bacteria for the production of biosurfactants. From the isolation result Bacillus Nealsonii was the identified microbe Raise (R) and it was used to for the production of biosurfactant, the broth was used as the nutrient source and kerosene was the carbon source. Then this biosurfactant was applied in the MEOR at room temperature (27°C) using a reservoir permeability tester equipment. The recovery process using this biosurfactant gave 68.42% of residual oil recovery after the primary and secondary oil recovery, thus these hyperthermophiles are good agents for MEOR.

1. Introduction

Production of crude oil has to undergo three levels (primary, secondary, and tertiary). throughout the process of primary recovery, the underlying crude oil is removed below natural pressure (because of natural gas) to force crude oil to the wellbore [1]. Secondary recovery is utilized to build recovery of crude oil when primary recovery is no more feasible, it occurs where a fluid (water or gas), the injection of crude oil into the reservoir is intended to maintain the reservoir pressure and to displace it towards wellbore [2-3]. There is still quite a lot of crude oil in the reservoir after primary and secondary processes are done. Then this leads to the tertiary or enhanced oil recovery (EOR) level.
EOR is a method for recovering oil that has not been extracted by the recovery methods of primary or secondary processes. EOR methods aim to extract more oil from reservoirs by various mechanisms such as interfacial reduction, wettability alteration, mobility control, physical property adjustment and gravity drainage [4]. The EOR process is classified into these categories: thermal, chemical (surfactants, polymers, etc.), microbiological, and miscible injection [5].

Conventional EOR processes require the utilization of chemical method; In chemical flooding processes, chemicals like surfactants, polymers are added to the injection brine to change the displacing fluid behaviour. Thermal methods; the principle behind thermal EOR is to utilize thermal energy to increase the reservoir temperature. This approach is especially useful for formations with a heavy or viscous hydrocarbon phase. Some of the factors involved in these processes are frequently limited by high chemical costs of and hazardous to the environment [6]. Therefore, an improved form of oil recovery using microorganisms, known as Microbial Enhanced Oil Recovery (MEOR) was introduced [7].

MEOR is a tertiary oil recovery methods employed to increase oil production efficiency [8]. This procedure utilizes microorganisms to enhance the recovery of crude oil entrapped in porous media [9]. Microorganisms associated with MEOR frequently generate biosurfactants, biomass, biopolymers, acids, solvents and gases which are of use in crude oil recovery operations [10]. It is also less expensive, more efficient, and environmentally-friendly [11].

Biosurfactant has proven to be an essential tool in MEOR procedures [12]. Biosurfactants are surface-active substances, particularly microorganisms gotten from living organisms [13]. They reduce surface tension, the interfacial tension between two fluids and improve residual oil [14]. Besides, biosurfactants can change the wettability of the rock formations. This modification causes relative permeability and alters it. This, in effect, will boost oil recovery by rising reservoir mobilization of the remaining oil [12]. Therefore biosurfactants are more environmentally friendly compared to conventional surfactants [15].

This study aims to isolate and identify potential biosurfactants producing bacteria from produced sand found inside a sandstone formation oil reservoir and its application.

2. Methodology

2.1. Materials

Autoclave, Centrifuge, Conical Flask (different size), Pipette, Sterile Tubes, Sterile Universal Bottles, Petri Plates, Weighing Balance, Kerosene, Incubator and Microwave.

2.2. Sample Collection

The reservoir sandstone sample was collected from an offshore platform in the Gulf of Guinea. The oil well has served as a platform for producing crude oil for about seven years. According to [16], the lithology of this subsurface is characterized by massive lithofacies mudstone, bedded muddy heterolith, shelly muddy sandstone and pebbly sandstone. Also, most of the reservoirs in the area are fairly consolidated sandstones. The sample sandstone was collected at a depth of 13, 657 ft via an in-situ treatment facility at the surface. The sand sample was of fairly uniform size and was placed in a plastic high-density container labelled with an indelible permanent marker before it was sent to Covenant University Biochemistry, Molecular Laboratory for analysis.

2.3. Isolation of Bacteria

The present study was carried at Molecular Biology Laboratory, Covenant University, Ota, Nigeria. The biosurfactants producing bacteria were isolated using the streak method on sterile Mueller Hinton agar
plates and at an optimum temperature of 37°C and duration of 24hrs it was incubated. The bacteria were streaked on new plates, when necessary and incubated for 24hrs at 37°C.

2.4. Identification of Biosurfactant Producing Bacteria

The 16S rRNA gene study was used in the genomic DNA of the selected biosurfactant producer was used to determine the taxonomic characterization of the strain. The DNA was removed by utilizing a commercial genomic DNA extraction kit (AidLab, China), as instructed by the manufacturer. Polymerase chain reaction (PCR) was conducted to amplify 16S rRNA gene found in the bacteria using the forward primer (27F: 50 AGAGTTTGATCCTGGGACGTA-30) and reverse primer 1492R (r50-GGTTACCTTGTGACTCTTAG-30). The PCR was performed in 20μl of a reaction mixture that contained 2.0μl of DNTP, 2.5μl of Taq buffer, 0.5μl of Taq polymerase, 0.5μl of 16S rRNA of forward primer, 0.5μl 16S rRNA of reverse primer and 2.5μl of DNA template. The PCR temperature profile was 5 minutes (1 cycle) at 95°C, 30 seconds at 94°C, 30 seconds at 52°C and 72°C for 30 seconds (30 cycles); and 72°C for 5 minutes following the final cycle. It was done on the C100 Touch Thermal Cycler BIO-RAD with a running process of 2.5hrs. Inqaba Biotechnical Industries (Pty) Ltd, South Africa, did the sequencing of purified products. The 16S rRNA gene nucleotide sequence of the isolate was compared with all deposited nucleotide sequences in the GenBank database using the BLAST system of the National Center for Biotechnology Information (NCBI). The alignments were checked using Geneious Prime (Version 2020.1) to construct a phylogenetic tree and to compare similarities among the sequences.

2.5. Production of Biosurfactant

The nutrient broth was prepared in 100ml of distilled water by dissolving the agar. The mixture was allowed to settle down and they sieved the supernatant out. It was sterilized at a pressure of 15 pascals and a temperature of 121°C, by autoclaving. The nutrient broth is poured into a sterile universal bottle after the solution cools down to room temperature. Inoculated into it, the bacteria were incubated at 37°C for 24hrs.

The production of biosurfactants was carried out using a mineral salt medium (MSM) consisting of 2.0 g/L KH₂PO₄, 5.0g/L K₂HPO₄, 3.0g/L(NH₄)₂SO₄, 0.1g/L NaCl, 0.01g/L FeSO₄·7H₂O, 0.2g/L MgSO₄·7H₂O, 0.01g/L CaCl₂·2H₂O and 0.002 g/L MnSO₄·H₂O, with the addition of kerosene (2%). 1000ml of distilled water was applied to it and autoclaved for 15 minutes at 121°C and 15psi. The broth had been poured into the MSM and incubated for 72hrs at 37°C.

2.6. Extraction of Biosurfactant

The cultures were centrifuged at the end of the fermentation at (12000 rpm for 30 minutes) to remove the cells. The residue was discarded, meanwhile, the cell-free supernatant was modified to pH 7 with 1M NaOH and the freeze-dried biosurfactants solution was weighed and placed at -7°C for the products obtained.

2.7. Flooding Experiment

Flooding experiment was done to estimate the influence of biosurfactants as a microbial enhanced oil recovery method. First water/brine was used to saturate the core. Subsequently, irreducible water saturation is calculated by injecting crude oil to replace the water/brine [17]. The biosurfactant solution was injected till no oil was observed and an incremental amount of recovery was noted and recorded.

3. Results and Discussion

3.1. Isolation of Bacteria
The result of the bacteria isolate is shown in figure 1. The process to obtain pure cultures was done using the streaking method. One colony (single) was picked up from the sample. The isolate (Raise, R) was sub-cultured into new plates to make it have abundant growth.

**Figure 1: Bacteria isolate**

### 3.2. Identification of Biosurfactant Producing Bacteria

The raw sequence data was gotten for the sample as a set of two sequences; one with forward and the other with reverse universal sequencing reaction primers as shown in figure 2. The Analyzed of raw sequence data was scrutinized to remove low-quality base calls using Geneious Prime (Version 2020.1). The closest sequence identities from the database were discovered by the BLASTn search of this nucleotide sequence with the most related 16S rRNA sequences from the Genbank database. The results obtained were most similar to the 99.02% similarity of *Bacillus Nealsonii*. The sequence was recorded under Accession number with GenBank: MT542325.

**Figure 2: Raw sequence data of the microbe.**

### 3.3. Production of Biosurfactant

The biosurfactant that emerged after 72hrs of incubation is shown in figure 3 below. According to [18] study, it was stated that most biosurfactants are considered as auxiliary metabolites and that some may assume a key part in the duration of microorganisms creating either by encouraging nutrient transport or microbe-host communications or as biocidal substances.

Foaming power is considered as one of the most significant effects that live in a biosurfactant obtained in the *Bacillus Nealsonii* supernatant. The medium’s steady foaming is seen as a qualitative measure of biosurfactants growth. It should be noted that the formation of foam in a mineral medium with kerosene as a carbon source represented a probable application of biosurfactants in MEOR during culture enrichment.
3.4. Flooding Analysis

The results presented in this chapter were given using the previously mentioned experimental procedures. This experiment had the aim of improving oil recovery by applying biosurfactants. The data and measurements given were performed at 27°C. Table 1 shows the Core Sample Characterization.

Table 1: Characterization of Core Sample.

| Core Sample | Length (cm) | Diameter (cm) | Pore volume | Porosity % | Absolute K (md) |
|-------------|-------------|---------------|-------------|------------|-----------------|
| Core R      | 3.2         | 3.5           | 19          | 0.29       | 248.15          |

Core sample (R) was put through waterflooding (brine injection). The water (brine) injection was performed at an injection rate of 1 cc/min from Reservoir Permeability Tester (RPT) to mobilize the oil and gain residual oil saturation.

After saturation of the residual oil, the oil produced through the injection cycle is controlled by taking note of the interface between oil and brine. The injection is performed until no more crude oil is created (interface-level changes). The study observed hereafter the core sample was flooded with the biosurfactants. Crude oil recovery was 68.42% after water flooding.
Table 2: Crude Oil Recovery Result Generated for Core R.

| Flooding type   | Time (mins) | Pore volumes (inj) | Pressure drop |
|-----------------|-------------|--------------------|---------------|
| Water flooding  | 5           | 0.13               | 13.6          |
| Water flooding  | 10          | 0.26               | 22.25         |
| Water flooding  | 15          | 0.39               | 23.35         |
| Water flooding  | 20          | 0.53               | 24.41         |
| Water flooding  | 25          | 0.66               | 25.54         |
| Water flooding  | 30          | 0.79               | 25.6          |
| Water flooding  | 35          | 0.92               | 27.1          |
| Water flooding  | 40          | 1.05               | 32.77         |
| Water flooding  | 45          | 1.18               | 34.33         |
| Water flooding  | 50          | 1.32               | 35.56         |
| Water flooding  | 55          | 1.45               | 36.1          |
| Water flooding  | 60          | 1.58               | 38.21         |
| Water flooding  | 65          | 1.71               | 39.87         |
| Water flooding  | 70          | 1.84               | 40.6          |
| Water flooding  | 75          | 1.97               | 41.23         |
| Water flooding  | 80          | 2.11               | 42.21         |
| Water flooding  | 85          | 2.24               | 42.69         |
| Water flooding  | 90          | 2.37               | 48.57         |
| Water flooding  | 95          | 2.50               | 47.34         |
| Microbial Surfc | 100         | 2.63               | 46.7          |
| Microbial Surfc | 105 | 2.76 | 46.3 |
|----------------|-----|------|------|
| Microbial Surfc | 110 | 2.89 | 45.1 |
| Microbial Surfc | 115 | 3.03 | 41.95 |
| Microbial Surfc | 120 | 3.16 | 43.1 |
| Microbial Surfc | 125 | 3.29 | 44 |
| Microbial Surfc | 130 | 3.42 | 45.6 |
| Microbial Surfc | 135 | 3.55 | 45.8 |

Figure 4 below shows the results of the volume of crude oil produced is calculated in cc against time. The findings show that water (brine) could no longer produce crude oil at 95 minutes, and some of the oil was left behind by bypass. During the flooding of water (brine) and biosurfactants, the core sample was flooded at an injection rate of 1 cc/min.

Figure 4: Oil recovery against time at 27°C

The relation between the biosurfactant recovery factor (%) as a function of the volume of the pore (a dimensionless parameter) in figure 5 below. The recovery is the ratio between the extracted volume of oil and the initial volume of oil as the injection volume pore is the ratio of the volume of fluid pumped into the reservoir. As shown in figure 5, it was discovered that after the initial injection of nearly 2.5 pore volume of water (brine), oil recovery stopped and water breakthrough began leaving some residual oil which could still be recovered. The more concentration is increased more oil will flow until it gets to the critical micelle concentration and stops producing. The flooding resulted in an additional 68.42% maximum recovery.
4. Conclusion

In this study biosurfactants producing bacteria were isolated and identified by using kerosene as sole carbon source. DNA extraction, PCR and sequencing were performed for the identification of the biosurfactants producing bacteria.

It was observed that the production of biosurfactants depends on the hours and as well as on incubation temperature. Further identification of isolate was done and it was found that the isolate was *Bacillus Nealsonii*. It had been grown to produce biosurfactants on the MSM medium. It is a power producer of biosurfactant that can be used in MEOR.

The flooding experiment carried out to examine the potential of biosurfactants as an agent for MEOR. Based on the experiment, the recovery process using this biosurfactant gave 68.42% of residual oil recovery after the primary and secondary oil recovery, thus these hyperthermophiles are good agents for MEOR.

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