Novel Acinetobacter sp. Isolated from Oil-contaminated Soil for Microbial Enhanced Oil Recovery

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ABSTRACT: The viscosity of hydrocarbon reservoirs increases with age. So, a significant amount of oil is trapped in the underground reservoirs after recovery using primary and secondary methods. To improve the recovery, tertiary methods have been used. However, there is limited research on Microbial Enhanced Oil Recovery (MEOR) as a tertiary method due to microbes' inability to survive the high-temperature reservoir conditions. Consequently, the goal of this research is the creation of a novel bacteria strain for MEOR. The isolated bacteria colonies were inoculated in a batch fermentation broth, and the biosurfactant produced was screened using the oil displacement, emulsification index, modified drop-collapse test, surface tension, and interfacial tension criteria. The effects of pH (7.2 – 10.52), and salinity (15 – 35 %) at optimum temperature were studied on the selected isolate, which was identified by partial 16 rRNA gene sequence analysis. Gas Chromatography-Mass Spectroscopy was used to characterize the biosurfactants produced. The new isolate reduced heavy oil viscosity by 17% and produced a recovery factor in the range of 13-16.72%. The new bacteria is a thermophile and survived at a temperature of 65 °C, indicating promise for use in MEOR.

KEYWORDS: Hyperactive bacteria, thermophilic bacteria, Oil Production MEOR, Biosurfactant production, bacteria screening, metabolites.

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I. INTRODUCTION

The microbial method for improving oil production has the potential to be a cost-effective and environmentally friendly method (Wu *et al.*, 2022). The existing field infrastructures can be used to reduce capital expenditure compared with the other Enhanced Oil Recovery (EOR) methods. High energy demand makes oil fields with moderate to high viscous oil difficult to recover. Matured fields are also associated with high paraffin deposition in the porous media, which reduces formation relative permeability and hinders oil mobility. For these reasons, a substantial fraction of oil remains trapped at the sub-surface after the primary and secondary recovery techniques (US Energy Information Administration (EIA), 2017). The primary oil recovery method uses the natural reservoir energy to extract 5–10% of the original oil. Secondary oil recovery requires injecting gas or water into the reservoir for pressure maintenance, and between 10 to 40% of the original oil is recovered (Hadia *et al.*, 2019)

Globally, between 2 to 4 trillion barrels of oil remain trapped in the sub-surface reservoirs due to capillary forces (Gao, 2018). They are being targeted for EOR, such as chemical, heat, and miscible displacement among others. The availability of gases such as carbon dioxide limits the miscible displacement EOR method. Other methods have been identified with various shortcomings, such as the damaging of down-hole equipment, permeability impairment, and low efficiency of heat utilization (Niu *et al.*, 2020). Microbial Enhanced Oil Recovery (MEOR) is a collection of techniques that utilize microorganisms and their metabolic products to improve oil production. The microorganisms produce gases and chemicals (acids, surfactants, gases, polymer, and biomass) by feeding on the oil as a carbon source. The production of these by-products in the reservoir can influence the changes in the reservoir fluid and rock properties

There are three major mechanisms involved in the MEOR(Saravanan *et al.*, 2020), the production of gases and acids that set free the trapped oil, the selective plugging of permeability channels which minimizes the by-pass oil, and the production of biosurfactants and polymers on the cell surface which modifies the rock preference wettability to oil and water. These mechanisms can, however, involve multiple biochemical and biophysical processes (Alkan, Mukherjee and Kögler, 2020). The field application of MEOR can be carried out in two ways depending on the sources of the microbes. If the indigenous reservoir microbes are stimulated and single species or consortia of naturally occurring bacteria are injected into the reservoir to produce specific metabolic products to improve oil recovery, the process is called *in-situ* MEOR (Gao *et al.*, 2016; Niu *et al.*, 2020). On the other hand, when microbes are made using traditional/conventional

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fermentation methods and deployed for MEOR, the process is *ex-situ* (Gao *et al.*, 2016). Regarding operation, field deployment of MEOR could be for cyclic or flooding. In the case of cyclic microbial recovery, the goal is to alter the drainage patterns and rock wettability near the wellbore to improve oil production rates. This technique may not only increase the ultimately recoverable oil in the reservoir but also increase cash flow and reduce the pay-back period (Al-Wahaibi, Grattoni and Muggeridge, 2006). In microbial flooding, on the other hand, crude oil properties and/or reservoir flow patterns are altered within the reservoir to aid in the mobilization of entrapped oil and increase the ultimate amount of oil production from the reservoir (Soudmand-asli *et al.*, 2007).

Metabolites such as bio-acids, biosurfactants, biopolymers, biogases, and bio-alcohols are produced by microorganisms and perform different functions when in contact with the trapped oils. For example, biosurfactants increase the mobility ratio of oil owing to their surface-active properties caused by the reduction of interfacial tension between crude oil and water, and the alteration of rock wettability (Aboelkhair, Diaz and Attia, 2022). There are four categories of surfactants, anionic, cationic, amphoteric, and non-ionic surfactants. The anionic groups are mostly preferred in EOR because of lower adsorption on reservoir rocks when compared to others. Several reports have indicated the effectiveness of surfactants in reducing the interfacial tension and alteration of rock wettability(Jha *et al.*, 2019; Pannekens *et al.*, 2019; Kumar *et al.*, 2020). Biopolymers increase the sweep efficiency by increasing water viscosity, thereby preventing water fingering(Hadia *et al.*, 2019; Soliman, El-hoshoudy and Attia, 2020). Bioacids dissolve certain rocks and open up their pores, thereby improving the interconnectivity of porous media and enhancing permeability(Soudmand-asli *et al.*, 2007). Biogas has two functions, which are decreasing the oil viscosity and increasing the reservoir pressure.

Up to today, there have been limited reports of MEOR field application in most hydrocarbon provinces despite many laboratory reports that have suggested its viability (Okoro *et al.*, 2022). In places where this technology has been applied, such as in China, the major challenge has been the adaptability of microbes to the subsurface temperature, toxicity, and salinity (Pannekens *et al.*, 2019; Ziwei *et al.*, 2021). This study was therefore desired to develop a novel thermophilic bacteria isolated from a Niger Delta-contaminated soil and evaluated it for MEOR.

II. MATERIALS AND METHODS

A. *Bacterial isolation*

A 5 g contaminated soil sample was placed in a 250 ml conical flask containing 50 ml of quarter-strength Ringer's solution (pH~7.0) and incubated at 23 °C on a shaker at 200 rpm for 7 days. The sample was serially diluted using quarter-strength Ringer's solution, streaked on mineral salts medium containing 1 % (v/v) sterilized crude oil as the sole carbon source and 1 ml per litre of trace element solution, and incubated for another 7 days. The medium pH was adjusted to 7 using 1 mol/L sodium hydroxide and hydrochloric acid, and the mixture was autoclaved at 121 °C for 15 minutes. The details of minerals, concentration, and procedure are available (Zhou *et al.*, 2015; Martzy *et al.*, 2019). After incubation, plates were enumerated, and different bacteria were purified on nutrient agar plates and then cultured at 37 °C for 24 h. The purified strains were stored on nutrient agar slants at 4 ± 2 °C for further analysis.

B. *Screening of bacterial isolates*

The screening was done to prune down the number of isolates and to select bacteria of biosurfactant production activity. To achieve this, the isolates were rejuvenated on nutrient agar plates for 24 h before inoculating in batches into a 5 ml fermentation broth. The fermentation broth consists of a mixture of solution A ($NaNO_3$ 2.5, NaCl 1.0, $MgSO_4.7H_2O$ 0.4, $CaCl_2$ 0.05, KCl 1.0, 85% H_3PO_4) g/L, solution B ($FeSO_4.7H_2O$ 0.5, $ZnSO_4.7H_2O$ 1.5, $MnSO_4.H_2O$ 1.5, K_3BO_3 0.3, $CuSO_4.5H_2O$ 0.15, $Na_2MoO_4.2H_2O$ 0.1) g/L and 2% glucose. The broth cultures were incubated under a shaker operated at 200 rpm for 10 days. All experiments were performed at 28 °C. After the incubation, each broth culture was centrifuged at 4,700 rpm for 10 minutes, and the cell-free supernatant was then screened for biosurfactant production as follows:

(i) Oil Displacement Test (ODT): 50 ml of distilled water and 20 µl of crude oil were mixed in a Petri plate (90 × 15 mm). A 10 µl aliquot of cell-free supernatant from fermentation broth culture was added to the surface of the oil, and the ODT was determined in triplicate following the (Morikawa and Hirata, 2000). In this method, a thin membrane was formed by adding 10 µl of crude oil sample (Niger Delta crude oil, Nigeria) to 40 ml of distilled water in a 150 mm diameter petri dish. Ten µl of 10 mM potassium phosphate buffer solution of pH 8.0 was carefully placed at the centre of the oil membrane. The area of the formed oil-displaced circle was measured as the activity of surfactants. The oil-displaced area formed by the activity of surfactants showed linear relations to the amount of surfactants tested.

(ii). Modified Drop-Collapse Test (MDCT):

The modified drop-collapse test was performed according to (Bodour and Miller-Maier, 1998). The test was conducted in a polystyrene lid with 96 round wells (inside diameter 8 mm). Initially, each lid was cleaned three times with hot water, ethanol, and distilled water before drying. After that, each well was coated with 1.8 µL of 10W-40 Pennzoil and spread thinly across the bottom. The coated wells were equilibrated for 24 hours to provide a consistent oil covering. A 5 µL aliquot of sample was delivered into the centre of the well using a 25 µL glass syringe (Hamilton, Reno, NV, USA), holding the syringe at an angle of 45°. The syringe was washed three times with water between each sample addition, followed by acetone. A standard curve was prepared for each surfactant by adding drops containing varied surfactant concentrations to each of the wells. At exactly 1 minute, the diameter of each drop was measured using a dissection microscope (153 magnification) with a calibrated micrometer. Droplets were observed at a standard time (1 min) to ensure consistent results.

Standard curves were made by plotting the graph of surfactant concentration against the drop diameter, which were used to determine surfactant concentrations in unknown samples. Samples were replicated five times and each experiment was repeated three times. Diluted hand-washing liquid, sodium dodecyl sulphate and distilled water served as control suspensions (Clien *et al.*, 2007).

(iii). Emulsification Index Test

The emulsifying capacity of culture samples was determined by mixing 2 ml each of kerosene and the cell-free supernatant and vortexed at 5000 rpm for 2 minutes. The mixture was allowed to stand for 24 and 72 hours, and E_{24} and E_{72} were estimated using Equation 1 (Campos, Stamford and Sarubbo, 2014).

$$H_{Emulsion} = \frac{H_{Emulsion\ layer}}{H_{Total\ mixture}} * 100\% \quad (1)$$

Where $H_{Emulsion}$ is the height of the emulsion layer, $H_{Total\ mixture}$ is the total height of the liquid.

(iv). Surface and Interfacial Tension Measurement

The cell-free biosurfactant from fermentation broth culture was subjected to surface tension measurement at room temperature (27±0.5 °C) using a Tensiometer (model K100). A sample was filled into a glass container and put in a measurement zone. After the measurement ring was inserted, it was zeroed in the air. The ring was then dragged down until it hung on the liquid's surface. To calibrate the apparatus, the surface tension of distilled water (blank experiment) was measured on different days. Then, the fine adjustment of the tensiometer was set to zero, and the measurement began and ended when the upward pulling force on the ring just balanced the downward force exerted by the fluid. Surface tension measurements were made in triplicates following the methodology of (Nasiri, 2014). For the Interfacial Tension (IFT), the procedure was the same as that of surface tension measurement, except that the measurement was carried out between biosurfactant and water / crude oil.

C. Identification of the selected bacterial isolates

The screened isolates were identified using partial 16S rRNA gene sequence analysis (Zhou *et al.,* 2015). The DNA was extracted using a simplified protocol based on the thermal lysis of bacterial cells (Boniek *et al.*, 2010; Martzy *et al.*, 2019). Polymerase Chain Reaction (PCR) was carried out using the universal bacterial primers 5F (5′ TGGAGAGTTTGATCCTGGCTCA 3′) and 531R (5′ TACCGCGGCTGCTGGCAC 3′) to obtain 16S rRNA gene amplicons. The biosurfactant was characterized using the GC-MS (Clarus 600 model and an Elite 5-MS) analysis technique.

D. Effects of pH and salinity on properties of biosurfactant at 65 °C

NGSI-A15 isolate which survived at 65°C was cultured at this temperature to determine the optimum pH and salinity for biosurfactant production. The test was conducted at pH and salinity at range (7.2 – 10.52) and (15 – 35 %), respectively.

III. EVALUATION STUDY

A. Design and description of the sand pack column for MEOR

A vertical sand pack column, 250 ml, is made of acrylic material equipped with two sieves fixed at the top and bottom of the column. The sieve caps were drilled to accommodate fluid in and out of the column. The sieve caps were protected using rubber rings to make it air and liquid-tight. The column was packed with well-sorted sandstone that had been autoclaved before use to eliminate any microbe that may be in the sand. The detailed design and geometry of the column are presented in Figure 1.

The volumetric calculation for evaluating the effects of biosurfactants in the sand pack was made using the recovery factor index. The estimates were made based on the residual oil after the water flooding of the column from a compactly designed gravity-assisted sand pack column.

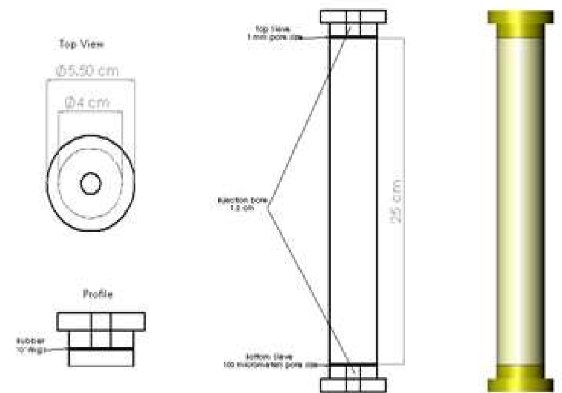

Figure 1: Cross section of the sand-pack column.

B. Effects of biosurfactant on heavy oil viscosity

Table 1 presents the physical characteristics of the heavy crude used for the analysis. The effects of different concentrations (50 - 100%) of the biosurfactant produced were examined at a pH of 7.20, salinity 15%, and 65 °C. The volumetric concentrations (%) of biosurfactant were determined by varying the volume of biosurfactant in a fixed volume of biosurfactant-water mixture. The step-by-step approach leading to the estimation of the recovery factor after the MEOR is described while the schematic representation of each of the procedures is shown in figure 2.

Step 1: Porosity determination.

The packed column was flooded with water through a suspended water storage equipped with an adjustable valve that releases water droplets by gravity until the sand is completely saturated with water. The volumetric flow rate after 60 seconds was determined (3 ml/min) and maintained throughout the experiment. The volume of water that saturated

the column was measured as the Pore Volume (PV). The matrix porosity (%) was calculated using Equation 2.

$$Porosity, \emptyset\ (\%) = \frac{PV}{Total\ volume\ of\ the\ column} * 100 \qquad (1)$$

Step 2: Original Oil In Place (OOIP

The heavy oil was injected into the water-saturated packed column and the water displaced was collected in a graduated cylinder. The process continued until there was no more water coming out from the effluent. The volume of oil injected was recorded as the OOIP, ml.

Step 3: Determination of fluid saturation

Initial oil saturation (S_{oi}, %) and initial water saturation (S_{wi}, %) were calculated using equations 2 and 3, respectively.

$$S_{oi}(\%) = \frac{OOIP}{PV} * 100 \qquad (2)$$

$$S_{wi}(\%) = \frac{(PV - OOIP)}{PV} * 100 \qquad (3)$$

For oil reservoir producing above bubble point, the sum of water and oil saturations is equal to unity as given in Eqn. 4.

$$S_{wi} + S_{oi} = 1 \qquad (4)$$

Step 4: Determination of residual oil saturation

The sand-pack column of known OOIP and S_{wi} was incubated at 65 °C for 24 h and afterward flooded with water. The volume of oil produced was collected and measured. The process was allowed to continue until no significant oil was produced and no traces of water were observed. The volume of oil produced after water flooding was measured and recorded as V_{Sorwf}, ml. The residual oil saturation (S_{or}) was calculated using Equation 5.

$$S_{or}(\%) = \left[\frac{(OOIP - V_{Sorwf})}{OOIP}\right] * 100 \qquad (5)$$

Step 5: Determination of recovery factor ($MEOR_{RF}$)

The column was flooded with water, and the volume of oil recovered was measured and recorded as V_{Sormf}, ml. The collected samples were centrifuged to break the emulsions formed. Different concentrations of biosurfactant produced were injected into the sand pack column containing the residual oil and the content allowed mixing (figure 2). The recovery factor using the MEOR (Rf_{MEOR}, %) was calculated after 10 days using equation 6.

$$Rf_{MEOR}, \% = \left[\frac{V_{Sormf}}{(OOIP - V_{Sorwf})}\right] * 100 \qquad (6)$$

All the experiments were performed in triplicate

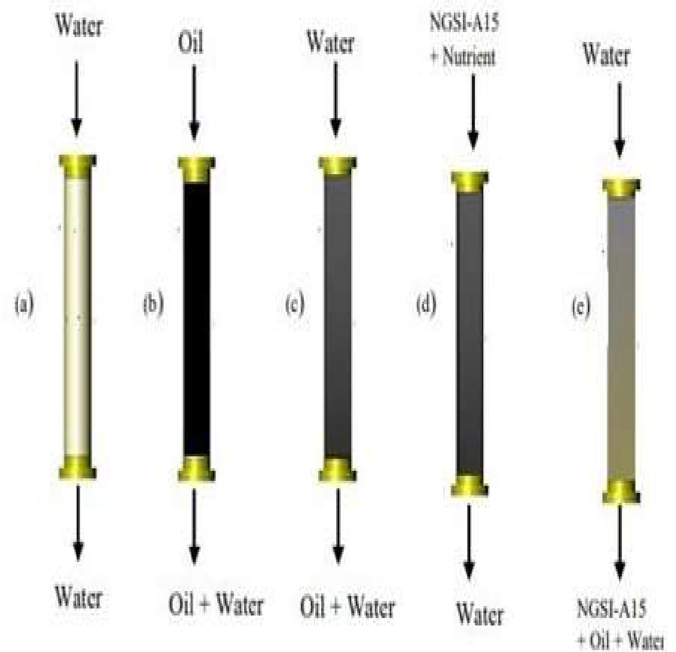

Figure 2: Schematic diagram for each of the steps (1 - 5) (a) column saturated with water (b) column saturated with Oil (c) residual oil saturation after water flooding (d) incubation with biosurfactant for 10 days at pH of 8.75, salinity of 15% and temperature of 65 °C (e) after residual oil production

C. Statistical analysis

All the data obtained for absorbance, ODT, E24, E48, E72, surface tension, interfacial tension, density, and viscosity were subjected to descriptive statistical analysis using Microsoft Excel spreadsheet. The effects of treatment with different concentrations of biosurfactant produced were analyzed by using analysis variance (ANOVA) using SPSS 7.0 software.

IV. RESULTS AND DISCUSSION

A. Bacterial isolates identification.

Table 1 presents the 16 bacteria isolated from the soil samples with their relative identity as obtained from the blast genetic sequence. Based on the percentage identity, blast search request ID, and nearness relative identity of the bacterial isolates it was observed that the isolates (NGS-BH2, NGSI-A15, and NGS-M19) and (NA-03, NA-19, NA-25, and NA-26) have the nearest identity typical of Acinetobacter sp., and Alcaligenes faecalis, respectively. The other isolates including NA-13, NA-24, NGS-M7, NGS-M9, NGS-M26, NA-12, NA-22, and NGS-M1 have nearest identities of providencia vermicola, providencia rettgeri, lysinibacillus fusiformis, flavobacterium sp., pseudomonas fluorescenes, Myroides indicus, Proteus mirabilis, and Psychrobacter alimentarius, respectively.

In Table 2, the result of the performance evaluation of biosurfactants produced by various isolates in the fermentation

broth at 45 °C and 7.2 pH was shown. The growths of the bacteria were measured by the absorbance of the biosurfactants produced at an optical wavelength of 550 nm (Liu, et al., 2011). According to (Elazzazy, Abdelmoneim and Almaghrabi, 2015), the higher the absorbance the higher the growth (population) of bacteria in a medium. It was observed that isolate NA-13 recorded the highest absorbance value (1.28 nm) while NA-19 exhibited the least (0.09 nm). At 45 ºC, the bacteria were observed to be very active. Conversely, NA-13 with the highest bacterial population had the lowest values of ODT (6.83 mm). The NGSI-A15 and NA-22 with lower populations exhibited higher values of oil displacement capacities of 13.42 mm and 13.40 mm, respectively. According to (Kurniati *et al.*, 2019; Saruni *et al.*, 2019; Ishag, Najib and Hamid, 2021)biosurfactants with high ODT contain certain metabolites that have the potential for oil mobilization.

As indicated in Table 2, all biosurfactants produced by the 16 isolates exhibited reduced ST and IFT in the fermentation broth compared to the control (water). Isolate NA-03 exhibited the highest value (58.17 dyne/cm) of IFT, while NGS-BH2 had the lowest value (50.00 dyne/cm). Therefore, all isolates can reduce ST and IFT at the interface between oil and biosurfactants (Gaol *et al.*, 2021). Surface and interfacial tensions between two immiscible phases can influence miscibility and relative mobility between phases (Berry *et al.*, 2015). The lower the IFT between the biosurfactant and oil, the higher the rate of absorption/mixing with crude oil. The degree of miscibility of biosurfactants with the oil determines the extent to which the oil viscosity is reduced (Gaol *et al.*, 2021). These surface forces often occur at the interface between water/oil and can affect the sweep efficiency.

Four (4) isolates (NA-03, NA-12, NA19, and NA-24) form no emulsion at 24 and 72 h. The remainder of the isolates showed various degrees of reduction of emulsification at 24 and 72 h. The NA-26 exhibited the highest E24 (42.86 %) while NA-13, NA-22, NGS-M7, and NGS-M9 showed the least (3.23%). The NA-13, NA-22, and MGS-M9 exhibited a reduction of emulsification (3.23 - 0.00 %) when emulsifying time increased to 72 h. The emulsification between E24 – E72 h for NA-26, NGSI-A15 and NGS-BH2 are (42.86 – 8.57 %), (33.33 – 1.56 %) and (33.33 - 16.67 %), respectively. This is evidence of the bioavailability and activity of the bacteria at tested conditions (Kaczorek, Olszanowski and Cybulski, 2005; Dastgheib *et al.*, 2008). About 75% of the emulsion formed with the biosurfactant produced by the isolates NGSI-A15 and NA-26 were observed to have broken down (demulsified), which shows also that the emulsion formed by these bacteria can be easily resolved.

B. Effects of salinity and temperature

It was shown in Figure 2 (a), the absorbance of the biosurfactants at 55 and 65 ºC. When the broth temperature was varied between 55 and 65 ºC, a significant reduction in absorbance was observed when compared to those measured at 45 ºC. However, the NGSI-A15 was observed to exhibit a high level of thermal stability within this range of temperature. At 65ºC for instance, only a 9.8% reduction of absorbance was noticed for the NGSI-A15 isolate the value which attested to its thermal stability. Similarly, NGSI-A15 produced a biosurfactant with the highest ODT (figure 2b), lowest surface and interfacial tensions (figure 2c), and good emulsification indices (figure 2d). The low surface and interfacial tensions are an indication of good surfactant production (Dastgheib *et al.*, 2008; Berry *et al.*, 2015; Gaol *et al.*, 2021).

All the isolated strains showed intolerance to high salt concentrations (>15%). This characteristic was earlier reported by (Shibulal *et al.*, 2014) and later by (Putra and Hakiki, 2019; Alkan, Mukherjee and Kögler, 2020). At 25, 30 and 35 % salinities, the biosurfactant produced by these isolates is undesirable for MEOR application for they clearly showed high values of ST and IFT, very low values of ODT and no emulsification values (E_{24} and E_{72}). At salinities in the range 15 - 20% however, the bioactivities of the microbes were observed to be restored when pH was varied between 7.2 and 10.52 for the broth temperature of 65 ºC. However, more gases, acids, alkalis, and surfactants were produced by the NGSI-A15 strain at a pH of 8.75. After multiple screenings, the NGSI-A15 - biosurfactant exhibited unique attributes that are comparable to those reported in the literature (Banat *et al.*, 2010; Makkar, Cameotra and Banat, 2011; Bachmann, Johnson and Edyvean, 2014). As shown in Figure 2c, only the NGSI-A15 strain formed emulsions (E24 and E72) at both 55 and 65 °C (figure 2d).

Table 1 Bacteria isolates and their relative identity from blasted genetic sequences.

Isolate Code	Blast Search Request ID	Sequence Length blasted (bp)	%Identity (Accession no.)	Nearest Relative
NGS-BH2	7GJHW10U014	634	94 (MF872586.1)	*Acinetobacter sp.*
NGSI-A15	7GNEVC5Y014	612	96 (MF872586.1)	*Acinetobacter sp.*
NA-03	7GPGGH1J014	555	99 (MG839276.1)	*Alcaligenes faecalis*
NA-13	7GS2BYA8014	627	96 (KX896673.1)	*Providencia vermicola*
NA-19	7GXNHHC8014	541	87 (LC361015.1)	*Alcaligenes faecalis*
NA-24	7GYN155B015	471	80 (MF098759.1)	*Providencia rettgeri*
NA-25	82N3336F014	736	100 (MG839276.1)	*Alcaligenes faecalis*
NA-26	82ND7M6R015	527	91 (MG839276.1)	*Alcaligenes faecalis*
NGS-M19	82NPXBA3015	716	97 (MF872586.1)	*Acinetobacter sp.*
NGS-M7	82PBNP2401R	660	92 (MG778880.1)	*Lysinibacillus fusiformis*
NGS-M9	82SCJX7B014	546	92 (MG788784.1)	*Flavobacterium sp.*
NGS-M26	82TC4Y7J015	540	85 (MF949058.1)	*Pseudomonas fluorescens*
NA-12	82U16524015	540	99 (NR_145659.1)	*Myroides indicus*
NA-22	82UEU8C0014	551	97 (CP026059.1)	*Proteus mirabilis*
NGS-M1	82UXTJ2E014	712	100 (CP014945.1)	*Psychrobacter alimentarius*

Table 2: Surface Active Properties of the Selected Bacterial Isolates Using Optimised Fermentatio Broth at 45°C and 7.2pH

Isolates	Absorbance (550 nm)	ODT (mm)	ST (dyne/cm)	IFT (dyne/cm)	E_{24} (%)	E_{72} (%)	Density (g/cm³)
NA-03	0.27 ± 0.18	6.33 ± 0.14	66.60 ± 0.36	58.17 ± 0.15	0.00	0.00	1.00
NA-12	0.34 ± 0.04	8.00 ± 0.13	64.47 ± 0.67	56.37 ± 0.35	0.00	0.00	0.98
NA-13	1.28 ± 0.30	6.83 ± 0.15	64.03 ± 0.06	56.17 ± 0.15	3.23	0.00	0.99
NA-19	0.09 ± 0.01	7.17 ± 0.13	65.63 ± 0.25	57.63 ± 0.25	0.00	0.00	0.99
NA-22	0.46 ± 0.02	13.40 ± 0.09	64.53 ± 0.47	56.10 ± 0.10	3.23	0.00	0.98
NA-24	0.24 ± 0.13	4.83 ± 0.04	63.27 ± 0.31	54.20 ± 0.10	0.00	0.00	0.99
NA-25	0.48 ± 0.13	9.00 ± 0.00	63.60 ± 0.35	54.17 ± 0.15	19.35	9.68	1.00
NA-26	0.74 ± 0.51	5.67 ± 0.05	62.57 ± 0.15	53.23 ± 0.25	42.86	8.57	0.98
NGS-M1	0.18 ± 0.06	5.67 ± 0.14	62.07 ± 0.06	52.57 ± 0.29	9.38	1.61	0.98
NGS-M7	0.49 ± 0.21	7.50 ± 0.12	61.43 ± 0.38	52.23 ± 0.15	3.23	1.61	1.00
NGS-M9	0.96 ± 0.22	9.00 ± 0.15	61.23 ± 0.21	52.53 ± 0.21	3.23	0.00	0.99
NGS-M19	0.35 ± 0.05	11.83 ± 0.12	66.70 ± 0.61	57.10 ± 0.10	16.67	3.33	0.99
NGS-M26	0.40 ± 0.29	7.08 ± 0.19	60.63 ± 0.31	50.23 ± 0.15	29.41	1.47	0.99
NGS-BH2	0.37 ± 0.12	6.67 ± 0.18	61.47 ± 0.31	50.00 ± 0.26	33.33	16.67	1.00
NGSI-A15	0.78 ± 0.19	13.42 ± 0.51	61.67 ± 0.25	55.30 ± 0.20	33.33	1.56	0.99
LC-14	1.17 ± 0.12	6.67 ± 0.08	60.27 ± 0.06	55.53 ± 0.21	15.63	6.25	0.99
Control (Distilled Water)	0.08 ± 0.00	-	72.53 ± 0.06	60.57 ± 0.06	0.00	0.00	1.00

C. Characteristics of NGSI-A15 - biosurfactant and its effects on crude oil

It was shown in Table 3 that the GC-MS composition of the crude biosurfactant produced by NGSI-A15 at different retention times. The chemical constituents of the biosurfactant produced by NGSI-A15 isolate through the GC-MS analysis revealed after 10 days of fermentation the presence of 2-pyrrolidinone, 2-piperidinone, acetic acid, pentanoic acid, 4-methyl-, ethyl ester, ethylene oxide, and methylene chloride. (Banat *et al.*, 2010) had reported these biosurfactants (2-pyrrolidinone, 2-piperidinone,) and other metabolites which are responsible for its performance at 65 °CpH 7.2 – 8.5, and 15 - 20% salinity.

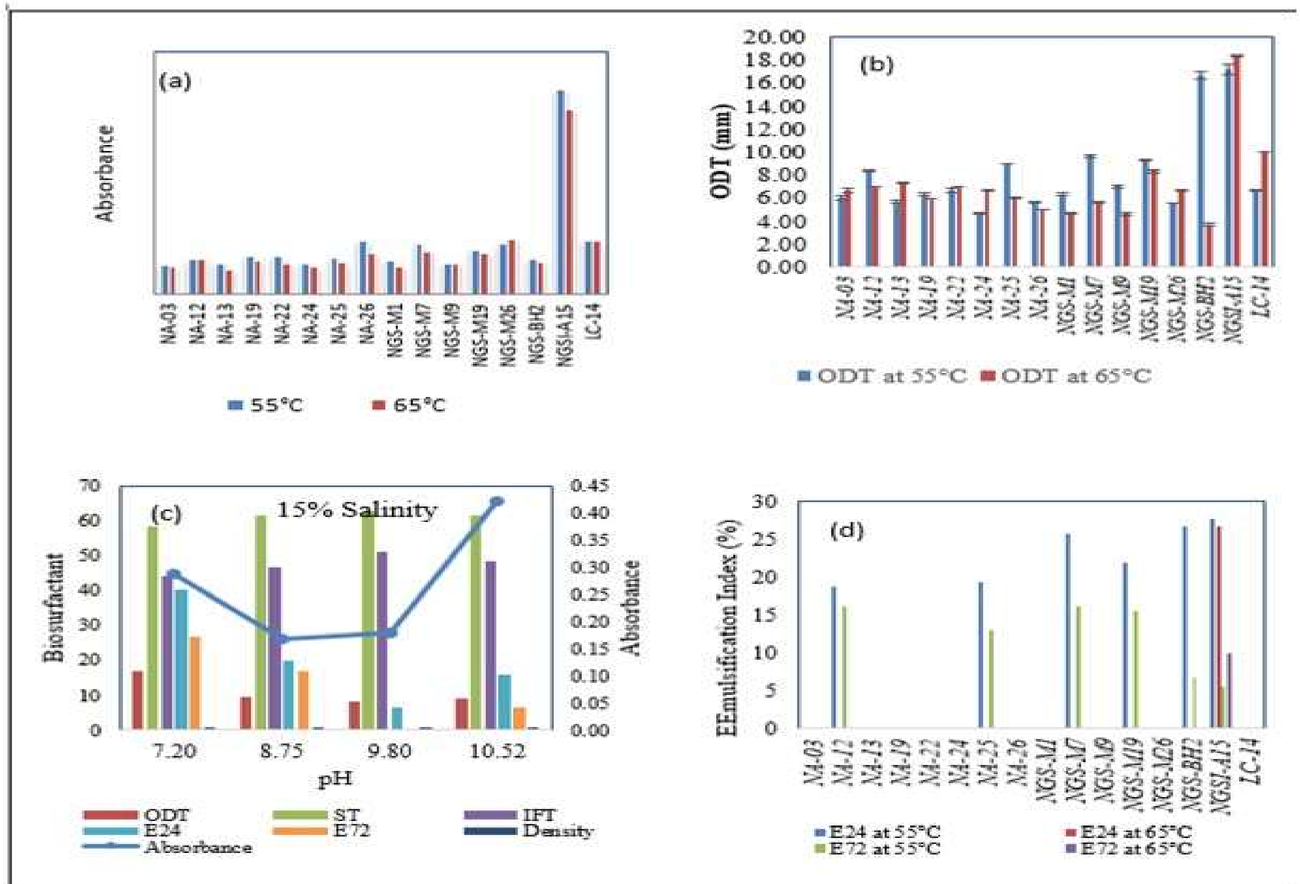

Figure 2: Effects of pH on quality of biosurfactants produced at 15%broth salinity and 65°C.

Table 3: Chemical Constituents of NGSI-A15 biosurfactant

	Retention Time (min.)	**Library Identity**	**Function**
NGSI-A15	1.275	Ethylene oxide or acetaldehyde	
	1.568	Methylene chloride	
	5.095	Acetic acid	
	8.136	Pentanoic acid, 4-methyl-, ethyl ester	
	9.561	2-pyrrolidinone	γ-lactam
	10.741	2-piperidinone	Lactam

The effects of different concentrations (0 – 100 v/v%) of the biosurfactant produced by NGSI-A15 at 0% salinity, pH of 8.75 and 65 °C on the ST, IFT, density, and viscosity of heavy oil are shown in Figure 3 while the effects of different concentrations at 15 % salinity, pH of 8.75 and 65 °C is shown in Figure 4. It is evidence that an increase in biosurfactant concentration leads to a decrease in oil viscosity. The oil viscosity was reduced from the initial value of 2.36 cp to 1.87 cp which corresponded to about a 20.8% reduction when the biosurfactant dosage was increased from 0 to 100 v/v%. The direct relationship of the volume fraction of biosurfactant/saline water was earlier reported by (Gudiña *et al.*, 2013) which attests to the consistency of the investigation carried out in this present study. Similarly, the density of the oil was observed to reduce as the biosurfactant volume fraction increased. Both the ST and IFT were found to decrease with increasing biosurfactant concentration up to 50% dosage beyond which there was no significant difference between ST and IFT

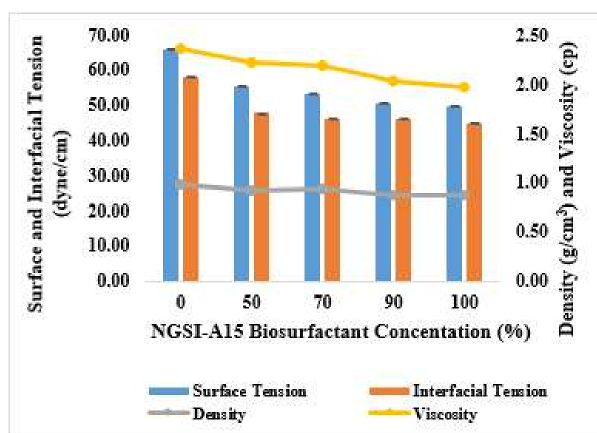

Figure 3: Effect of Different Biosurfactant Concentrations on Heavy Crude (100%)

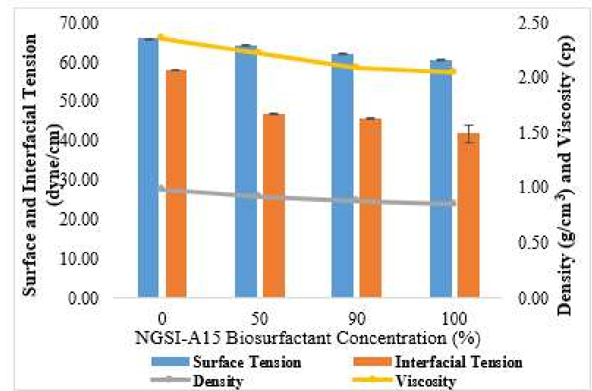

Figure 4 Effect of different biosurfactant concentrations on the mixture of heavy crude (45%) and saline water (55%)

D. Performance evaluation

It was shown in Table 4 the result of the sand porosity, saturation, and volumetric balance of the fluid in place. The result of the recovery factor obtained after the waterflooding of the oil-saturated sand pack using biosurfactant produced by the NGSI-A15 isolate at 65°C and pH of 8.75 for different salt/biosurfactant volume ratios is presented in Table 5. The amount of residual oil recovered after microbial flooding varied depending on the volume fraction of the biosurfactant used. These differences are most probably due to the reduction in viscosity of the entrapped oil in the column by the surfactant. There was a significant difference in the values of recovery due to biosurfactant (Vs_{ormf}) when compared to the control. But as the volume fraction of the biosurfactant increases (50 – 100 v/v%), more entrapped oil is swept efficiently due to the reduction in viscosity, density, surface, and interfacial tension (She, et al., 2011). The increment in production due to biosurfactant is in order of control < 50 v/v < 70 v/v < 90 v/v < 100 v/v. The highest biosurfactant volume fraction exhibited the highest production with a recovery factor of 16.72 ± 0.02%. Table 6 compared the performance of the NGSI-A15 (*Acinetobacter sp.*) with bacterial strains reported by some authors which ranged between 5 – 22%. In this present study, the evaluation was performed at a higher temperature, and the recovery factor in the range of 13–17 % recorded shows that NGSI-A15 has potential application in MEOR.

Table 4: Rock properties and volumetric balance

Bulk vol. (ml)	Pore vol. (ml)	φ (%)	S_{wi} (%)	S_{oi} (%)	OOIP (ml)	V_{Sorwf} (ml)	V_{Sormf} (ml)
250	98.11 ± 0.03	39.2 ± 0.01	9.37 ± 0.01	90.63 ± 0.00	88.94 ± 0.01	60.89 ± 0.00	28.03 ± 0.01

Table 5: Oil physical properties and post-waterflooding recovery factor

	Control	50 (v/v%)	70 (v/v%)	90 (v/v%)	100 (v/v%)
$V_{MEOR}(ml)$	1.21± .01^{a}	3.59±0.00^{b}	3.81±0.02^{b}	4.38±0.01^{c}	4.67±0.01^{c}
$Rf_{MEOR}(\%)$	4.32± .00^{a}	12.81± 0.00^{b}	13.58±0.0^{c}	15.64 ±0.00^{d}	16.72±0.00^{e}
$Density\ (g/cc)$	0.98 ± 0.01^{a}	0.93 ± 0.02^{a}	0.93 ±0.02^{a}	0.87 ± 0.01^{b}	0.87 ± 0.00^{b}
$Viscosity\ (cp)$	2.36 ± .01^{a}	2.22 ± 0.02^{a}	2.20 ± .02^{a}	2.04 ± 0.02^{b}	1.96 ± 0.02^{c}

Values are means of triplicates
Values along the same row having different letters (a, b, c, and d) indicate that they are significantly different ($p<0.05$).

Table 6: Comparison of Rf from this study with the literature

Microorganism	Recovery factor (%)	Reference
NGSI-A15 (*Acinetobacter* sp.)	12.81 – 16.72	This study
Halanaerobium	5.3 - 29.6	(Kögler *et al.*, 2021)
B, licheniformis XDS1, XDS2, *Bacillus cereus* XDS3	4.80– 6.90	(She *et al.*, 2011)
B, licheniformis BNP29	9.30 – 22.10	(Yakimov *et al.*, 1997)
Bacillus brevis, Bacillus polymyxa, B, licheniformis	18.00	(Almeida *et al.*, 2004)
B, licheniformis AC01	21.70	(Dastgheib *et al.*, 2008)

V. CONCLUSION

A new bacterial (NGSI-A15) was isolated in this study from oil-contaminated soil within the Niger Delta, Nigeria. The isolate is closely related to *Acinetobacter sp* according to DNA sequence analysis with a nearness relative identity of 96%. NGSI-A15 has shown promising potential as a biosurfactant producer for MEOR when tested after 10 days of fermentation in sand packs at 65 oC, pH of 8.75, and water salinity of 15%. The recovery factor RFMEOR, an indication of an incremental production after waterflooding the oil-saturated unconsolidated sand packs ranged between 12.81 – 16.72%. This figure is attributed to the presence of 2-pyrrolidinone (γ-lactam) and 2-piperidinone (lactam). Members of the *Acinetobacter sp* could be a promising MEOR target species for MEOR.

AUTHOR CONTRIBUTIONS

S. E. Agarry: Conceptualization, **A.O. Arinkoola:** Supervision, Writing – original draft, Writing – review & editing. **S.B. Akinde:** Supervision, Methodology, Laboratory, **L. M. Rafiu:** Methodology, Laboratory, Writing – original draft, Validating, **G. K. Latinwo, E. O. Dada, S. O. Alagbe, A.G. Akinyemi, I. A. Oladunjoye, R. A. Adepoju:** Software, Laboratory and editing.

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