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Evaluation of Microbial Enhanced Oil Recovery Potential of Biosurfactants from a Thermo-tolerant Bacterium in Sand Packed Columns

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Abstract: Microbial Enhanced Oil Recovery (MEOR) is a collection of techniques that utilize microorganisms and their metabolic products to improve the recovery of oil. The microorganisms produce gases and chemicals (bio-acids, bio-surfactants, bio-gases, bio-polymer and bio-mass) with oil as carbon source. The production of these by-products in the reservoir changes the reservoir fluid and rock properties and consequently improves the sweep efficiency which in turn increases the oil production. Previous researches worked on the use of biosurfactant to reduce surface properties of water at mild environmental conditions while this work worked on a thermo-tolerant isolate NGSI-A15 and its metabolite (biosurfactant) at 1.5 % salinity, pH of 7.2 and temperature of 65°C on surface properties of (45-55 %) oil/water mixture and 100 % oil as well as additional oil recovery in sand packed column. NGSI-A15 under extreme environmental conditions was able to solve problems of high viscous, high density and stable emulsion encountered in crude oil exploration having showing capacity to reduce heavy crude oil viscosity, density, ST and IFT. The bacterial strain, NGSI-A15 (*Acinetobacter sp.*) shows some improvement of 13–17 % of additional oil recovery (AOR) in sand packed column. With these properties, the biosurfactant produced is capable of detaching and mobilizing the trapped oil in an oil well (reservoir). The isolate, NGSI-A15 under high environmental conditions has the capacity to solve problems of high viscous, high density and stable emulsion encountered in crude oil exploration having showing capacity to reduce heavy crude viscosity, density, ST and IFT.

Keywords: *Microbial Enhanced Oil Recovery, Biosurfactants, Vertical Sand Pack Column, Original Oil in Place, Additional Oil Recovered.*

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I. INTRODUCTION

Microbial methods for improving oil recovery are potentially cost-effective and well-suited for today's economic climate (Alkan, *et al.*, 2023). They require little capital expenditure and are easily implemented in the field (She, *et al.*, 2019). The majority of oil fields in the Niger Delta basin are characterized by high content of paraffin with exceptionally high pour and cloud points. These conditions cause paraffin deposition in the porous media, which reduces permeability and can hinder fluid transportation via pipelines (She, *et al.*, 2019; Alkan, *et al.*, 2023; Soudmand-Asli *et al.*, 2007).

The primary and secondary recovery technologies are limited only to 35-65 % of Original Oil In Place (OOIP) (Geetha, *et al.*, 2018). Consequently, a substantial volume of oil is trapped within the reservoir interlayers, lenses and stagnant zones. Producing these oils poses serious challenges that become more complicated as the reservoir becomes more heterogeneous (Alkan, *et al.*, 2023). Globally, it has been reported that about 2 to 4 trillion barrels of oil is trapped in the sub-surface reservoirs that are being targeted for Enhanced Oil Recovery (EOR) technologies (Wang, *et al.*, 2019, Nmegbu, 2014). The most widely used EOR techniques have been the use of chemical, heat and miscible displacement (Udipta, *et al.*, 2013, Alkan, *et al.*, 2023).

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However, these methods have inherent shortcomings. The miscible displacement method is limited by the availability of gases such as carbon dioxide and corrosion of pipelines (Chen, *et al*., 2022, Chotard, *et al*., 2022). Other shortcomings include high cost of surfactant, incessant polymers breakdown, emulsion blockage, damages of down-hole equipment, permeability impairment and low efficiency of heat utilization (Udipta, *et al.*, 2013, Chotard, *et al*., 2022). The concept of the use of microorganisms to recover oil from the porous media is dated back to 1926 (ZoBell, 1947). Since then numerous studies have concentrated efforts only to demonstrate its effectiveness at laboratory scale.

Microbial Enhanced Oil Recovery (MEOR) is a collection of techniques that utilize microorganisms and their metabolic products to improve the recovery of oil (Rafiu, 2019, Alkan, *et al.*, 2023, Rafiu, *et al*., 2024). The microorganisms produce gases and chemicals (acids, surfactants, gases, polymer and biomass) with oil as carbon source. The production of these by-products in the reservoir changes the reservoir fluid and rock properties and consequently improves the sweep efficiency which in turn increases the oil production. This process involves three major mechanisms. The production of gases and acids which sets free, the trapped oil, selective plugging of permeability channels and production of biosurfactants and polymers on the cell surface. These mechanisms can be quite complex and involve multiple biochemical and biophysical processes (Chen, *et al*., 2022, Udipta, *et al.*, 2013, ZoBell, 1947). Indigenous reservoir microbes are usually stimulated and single species or consortia of naturally occurring bacteria can be injected into the reservoir to produce specific metabolic products to improve oil recovery (Udipta, *et al.*, 2013, Chotard, *et al*., 2022). In other applications, *ex-situ* made microbes by traditional fermentation have been successfully deployed for MEOR. Basically, MEOR applications can be by either cyclic or flooding. The goal of cyclic microbial recovery is to alter the drainage patterns and rock wettability near the wellbore to improve oil production rates. This technique may not only increase the ultimate recoverable oil in the reservoir but also increase cash flow and reduces the pay-back period (Al-Wahaibi, *et al.*, 2006, Alkan, *et al.*, 2023, Rafiu, *et al*., 2024). The goal of microbial flooding is to alter crude oil properties and/or reservoir flow patterns within the reservoir to mobilize entrapped oil and increase the ultimate amount of oil recovered from the reservoir (Soundmand-asli, *et al.*, 2007, Chen, *et al*., 2022).

Notwithstanding, there have not been any report of MEOR method in the Niger Delta hydrocarbon fields. However, an approach to apply the technology should consider primarily: microbiological studies to select the appropriate microorganisms and mobilization of oil in laboratory experiments to ascertain its effectiveness before field application (Rafiu, 2019, Rafiu, *et al*., 2024).

The aim of this study is to evaluate the potential of biosurfactants obtained from a thermo-tolerant bacterium isolated from a Niger Delta contaminated soil for additional oil recovery in sand packed columns for microbial enhanced oil recovery.

II. MATERIALS AND METHODS

➢ *Determination of Biosurfactant Activity of NGSI-A15 on Heavy Crude*

The supernatant produced when NGSI-A15 isolate was subjected to the selected optimum conditions (pH and salinity) at 65 °C was serially diluted with sterile distilled water to make up 1ml of the mixture. The following volumes: 500, 700, 900 and 1,000 µl of biosurfactant was serially diluted with sterile distilled water to make up 1ml of the mixture and added to 5 ml of heavy crude. Thereafter, another volumes biosurfactant: 500, 900 and 1,000 µl also was serially diluted with sterile distilled water to make up 1ml of the mixture was added to heavy crude (45 %) and saline water (55 %). All were added in a glass tube (25 x 500 mm).

The heavy crude (see Table 1 for its properties) was pre-sterilized by autoclaving at 121 °C for 15 minutes before used. The mixture was vigorously missed in a vortex mixer at 4,000 rpm for 10 minutes and subsequently incubated on shaker incubator at 65 °C for ten (10) days. The biosurfactant activity of the NGSI-A15 isolate on the properties of heavy crude was measured by the determination of the density, viscosity, surface tension and interfacial tension. This experiment was conducted in triplicate with the mixture of sterile heavy crude and distilled water without biosurfactant served control.

Table 1: Properties of the Hydrocarbon Mixtures

Hydrocarbon mixture	η (mPa s)	ρ (g cm^{-3})	API	n-alkanes range
Heating oil	1.34	0.76		C16–C30
Viscous paraffin	44.64	0.85		
Arabian light oil	8.33	0.86	29.7°	C11–C24
Heavy crude oil	73.91	0.90	25.5°	C14–C32

Viscosity (η), density (ρ), API gravity (API) and n-alkanes range

➢ *Effect of Biosurfactant on MEOR in Sand-Pack Column*

The oil well reservoir simulation was performed by the use of a designed vertical sand-pack column at the University of Benin, Nigeria. The sand pack was utilized for the evaluation of the performance of biosurfactant produced by NGSI-A15 isolate on additional oil recovery (AOR). The design of the column was vertical sand pack column (VS-PC) as conceived by Gudiña, *et al.*, (2013). Rafiu, *et al.*, (2024) applied the column, the sand pack was made of acrylic column positioned vertically with a volume of 250 ml and has two sieves and fixed at both ends of the cylindrical column. The bottom sieve was positioned and the cap fixed. They were uniformly packed with autoclaved dry sand to eliminate any microbe that may be in the sand. After packing the sand

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tightly, the top sieve and cap were fixed. The caps on both the ends of the column were provided with holes for insertion of in-flow and out-flow tubes. Rubber 'O' rings surrounded the caps to hermetically seal the column to ensure air and liquid tight (see Figure 1 for detail description).

The experiment was carried out at different concentrations (0, 50, 70, 90 and 100 %) of the biosurfactant produced by NGSI-A15 on the recovery of heavy crude oil in the sand-packs. The experiments were carried out at pH of 7.20, salinity 1.5 % and 65 °C. The heavy oil (see Table 3.1 for properties) collected from a well in Port Harcourt, in the Niger Delta of Nigeria was used for the evaluation. A schematic representation of the process is shown in Figure 2 and a brief description is provided as follows:

(1). The column was flooded first with water at a constant flow rate of 3 ml/min. Pore volume (PV, mL), which represents the empty volume of the model, was calculated by measuring the volume of water used for saturating the column. The matrix porosity (%) of the column was calculated as the PV divided by the total volume of the column (250 ml).

(2). In the second step, in the same way the heavy oil (previously sterilized) was injected into the column to replace water, until there was no more water coming out from the effluent. Original oil in place (OOIP, ml) was calculated as the volume of oil (heavy crude) retained in the column. Initial oil saturation (S_{oi}, %) and initial water saturation (S_{wi}, %) were calculated using equations 3.1 and 3.2 (Eduardo, *et al.*, 2013):

$$S_{oi}(\%) = \frac{OIIP}{PV} * 100 \qquad 3.1$$

$$S_{wi}(\%) = \frac{(PV - OIIP)}{PV} * 100\ \% \qquad 3.2$$

(3). The sand-pack column was incubated at 65 °C for 24 h and afterwards flooded again with water to remove the excess of oil, until no more of it (oil) was observed in the effluent (out-let flow tube). The amount of oil recovered (oil recoverable after water flooding (S_{orwf}, ml)) was determined volumetrically. Its (column) residual oil saturation (S_{or}) was calculated as follows:

$$S_{or}(\%) = \left[\frac{(OIIP - S_{orwf})}{OIIP}\right] * 100 \qquad 3.3$$

(Gudiña, *et al.*, 2013)

(4). The residual oil was subjected to microbial enhanced recovery processes. During which the column was flooded with different concentrations of biosurfactant produced by NGSI-A15 isolate in fermentation broth (FB) supplemented with glucose (FB medium) and incubated for 10 days at pH 7.2, salinity 1.5 % and 65 °C. Control columns were inoculated only with FBand incubated at the same conditions (Gudiña, *et al.*, 2013).

The fermentation broth was a mixture of solution A (1,000 ml), solution B (3 ml) and 2% glucose as the sole carbon and energy source A (g/l): $NaNO_3$ 2.5, NaCl 1.0, $MgSO_4.7H_2O$ 0.4, $CaCl_2$ 0.05, KCl 1.0, 85% H_3PO_4 10 ml adjusted to pH 7.2 with KOH]. [Solution B (g/l): $FeSO_4.7H_2O$ 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] (Bodour, *et al.*, 2003; Bodour and Miller-Maier, 1998).

This medium contains nitrate (ammonium nitrate) which can be used as an alternative electron acceptor by the microorganism isolate whenever growing under anaerobic or oxygen limiting conditions. This is important because, although the conditions inside the sand-pack columns are not strictly anaerobic, the low amount of oxygen present should be quickly consumed by the microorganisms.

(5). The column was flooded with water and the volume of heavy oil recovered (oil recovered after microbial flooding (S_{ormf}, ml)) was measured volumetrically. The samples were centrifuged to break the emulsions formed. Enhanced Oil Recovery (EOR, %) was calculated as follows:

$$EOR, \% = \left[\frac{(S_{ormf})}{(OIIP - S_{orwf})}\right] * 100 \qquad 3.4$$

(Gudiña, *et al.*, 2013)

All the experiments were performed in triplicate

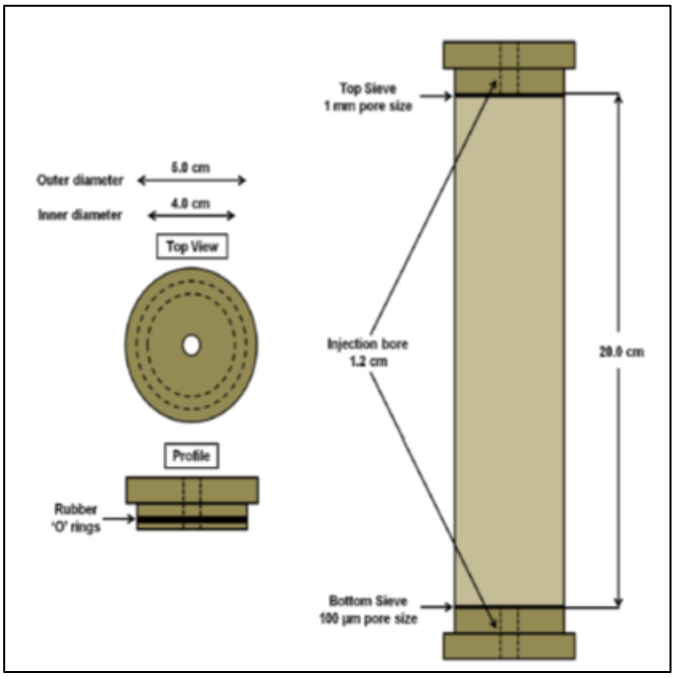

Fig 1: Design of the Sand-Pack Column Model Used to Evaluate the Mobilization of Residual oil by Microorganisms (Gudiña, *et al.*, 2013).

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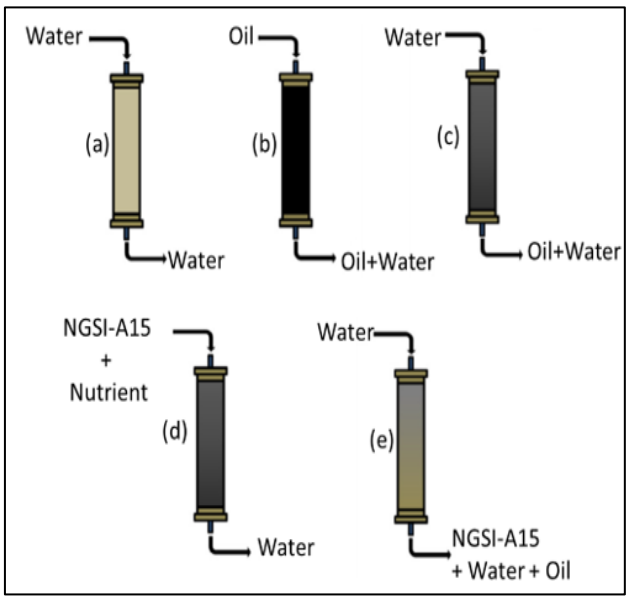

Fig 2: Schematic Representation of the Sand-pack Column Process (a) Column Saturated with Water (b) Column Saturated with Oil (c) Residual Oil Saturation After Water Flooding (d) Incubation with Biosurfactant (NGSI-A15) Isolate After 10 days at 65 °C (e) Additional Oil Recovery after NGSI-A15 flooding (Gudiña, *et al.*, 2013).

III. RESULTS AND DISCUSSION

- *Effects of Biosurfactant Produced by NGSI-A15 Isolate on Heavy Crude Oil*

The effects of different concentrations of the biosurfactant produced by thermophilic bacterial isolate(NGSI-A15) at 1.5 % salinity, pH of 7.2 and temperature of 65 °C on 100 % heavy petroleum oil surface properties were evaluated and presented in Figure 3 and 4 respectively. The properties were measured by surface and interfacial tensions (dyne/cm), density (g/cm^3) and viscosity (centipoises) as presented in figures. In Figure 3, the viscosity of the heavy oil decreases as biosurfactant concentrations increases. The most significant viscosity reduction was observed at 100% biosurfactant concentration. There were significant differences in the values of viscosities between the control and that of biosurfactant concentrations. The highest value of viscosity was attained with 50 % biosurfactant concentration. There is little or no significant difference in the values of densities obtained for each concentration of biosurfactant produced but yet, the density decreased as concentration of biosurfactant increased. The surface and interfacial tensions values were low for the different biosurfactant concentrations. The most significant low values of ST and IFT were obtained at 100 % biosurfactant concentration. However, the biosurfactant produced by NGSI-A15 at 1.5 % salinity, pH of 7.2 and temperature of 65 °C displayed characteristics of a good surfactant as reported by Sudhaker, et *al.*, (2015), Cholard, *et al*., (2022).

Figure 4. shows effects of different concentrations of the biosurfactant produced by thermophilic bacterial isolate (NGSI-A15) at 1.5 % salinity, pH of 7.2 and temperature of 65 °C on surface properties were evaluated on mixture of 45 % heavy petroleum oil and 55 % saline water to simulate oil/water reservoir composition. Across the biosurfactant concentrations, the heavy oil viscosity and density values recorded little decrease as biosurfactant concentration increases. 100 % biosurfactant recorded highest reductions in viscosity with 13.34 % and highest density reduction of 13.27 %. 100 % biosurfactant concentration recorded least value of ST with 60.73 dyne/cm while the highest value of ST was observed at 50 % biosurfactant concentration with 64.30 dyne/cm. There was a sharp reduction in the value of IFT recorded on addition of 50 % biosurfactant concentration from 58.13 dyne/cm (control) to 46.80 dyne/cm. No significant difference in the values of interfacial tension recorded across the biosurfactant concentrations (50 %, 90 % and 100 %) added to the mixture. The biosurfactant produced by this isolate (NGSI-A15) had displayed quality of a good surfactant having exhibited the capacity to reduce the values of IFT of oil/saline mixture and viscosity of the oil in the mixture. With these properties, the biosurfactant produced is capable of detaching and mobilizing the trapped oil in an oil well (reservoir). This isolate, NGSI-A15 under extreme environmental conditions was able to solve problems of high viscous, high density and stable emulsion encountered in crude oil exploration having showing capacity to reduce heavy crude viscosity, density, ST and IFT.

It has demonstrated capability to de-emulsify the emulsion formation which can take care of high stable emulsion and high viscosities that are facing oil exploration. Therefore yield stresses will be alleviated and more crude oil will be librated and available for exploration (Devi and Bhagobaty 2021, Ronningsen, 2012 and Ainon, *et al.*, 2013).

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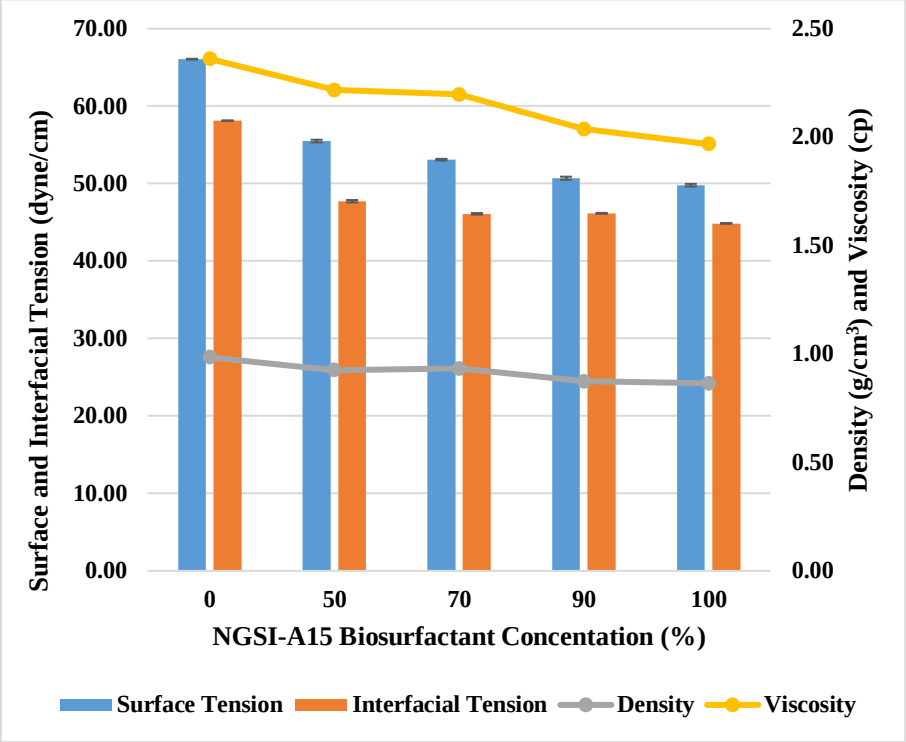

Fig 3: Effect of Different Biosurfactant Concentrations on Heavy Crude (100%)

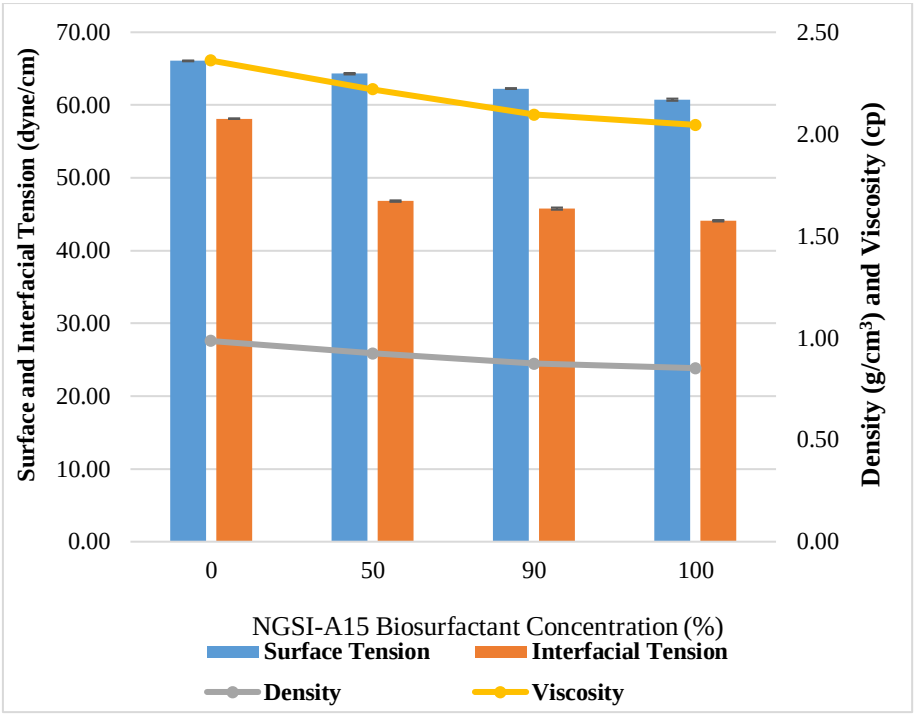

Fig 4: Effect of Different Biosurfactant Concentrations on Mixture of Heavy Crude (45%) and Saline Water (55%)

➢ *Application of Biosurfactant Produced by NGSI-A15 Isolate for MEOR*

The results obtained on the effect of different biosurfactant concentrations (50-100 %) produced by NGSI-A15 isolate are presented in Table 1 and 2. Since the sand–pack column has total volume of 250 ml, the pore volume of 98.11±0.01 ml and the porosity of 97.70±0.01 % of the packed column were estimated. The OOIP value recorded was 98.50±0.02 ml. The initial oil and water saturations recorded are 90.63±0.04 and 9.37±0.02 ml respectively. The amount of oil recovered after water flooding process (S_{orwf}) was 25.20±0.03 ml and the residual oil saturation (S_{or}) in the column was 60.89±0.03 ml.

The amount oil recovered after microbial flooding (S_{ormf}, ml) varied depending on the biosurfactant concentrations used as shown in Table 2. These differences are most probably due to the reduction in viscosity of the

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entrapped oil in the column by the biosurfactant concentrations. Therefore, there was significant difference in the values of S_{ormf} between the control and biosurfactant concentrations.

As concentration of the biosurfactant flooding increases, more entrapped oil got librated due to reduction in viscosity, surface and interfacial tensions as well as density reduction and surface wettability alteration potential of the biosurfactant. These were manifested in the values of oil recovered after microbial flooding (S_{ormf}) and additional oil recovered (AOR) as presented in the Table 2.

The additional oil recoveries recorded for all cases when compared to the control set-up indicated the concentration variation as dominant parameter affecting viscosity reduction for the heavy petroleum crude. The highest biosurfactant concentration (100 %) used exhibited most effective in AOR with value of 16.72±0.02% and 50% recorded least AOR value of 12.81±0.02. The least concentration (50 %) recorded significant difference value of AOR compared to control.

However, this bacterial strain, NGSI-A15 (*Acinetobacter sp.*) shows some improvement (13–17 % of AOR) in performance above bacterial strains reported by some authors as shown in Table 3. The reported AOR values by different authors were based on different hydrocarbon grades at temperature below the 65 °C used in this study and their AOR values reported ranged approximately between 5–22 %.

Table 1: Results Obtained in the Sand-Pack Experiments After Water Flooding

Total Vol. (ml)	Pore Vol. (ml)	Porosity (%)	OOIP (ml)	S_{oi} (%)	S_{wi} (%)	S_{orwf} (ml)	S_{or} (%)
250	98.11±0.01	97.70±0.01	98.50±0.02	90.63±0.04	9.37±0.02	25.20±0.03	60.89±0.03

Table 2: Results Obtained in the Sand-Pack Experiments After Biosurfactant Flooding (MEOR)

	Control	50%	70%	90%	100%
S_{or} (%)	60.89±0.03	60.89±0.03	60.78±0.02	60.69±0.02	60.77±0.01
S_{ormf} (ml)	2.80±0.04	8.30±0.03	8.80±0.02	10.20±0.05	10.90±0.02
OOIP-S_{ormf}(ml)	64.80±0.01	64.80±0.04	64.80±0.04	65.20±0.03	65.20±0.02
AOR (%)	4.32±0.02	12.81±0.02	13.58±0.01	15.64±0.03	16.72±0.02
Density(g/cm3)	0.98±0.00	0.93±0.01	0.93±0.00	0.87±0.01	0.87±0.00
Viscosity (cp)	2.36±0.01	2.22±0.01	2.20±0.00	2.04±0.01	1.97±0.00

Table 3: Comparative of this Study with AOR Reported in the Literatures

Microorganism	Substrate	AOR (%)	Reference
NGSI-A15 (*Acinetobacter* sp.)	Heavy crude oil	12.81 – 16.72	This study
B, licheniformis XDS1, XDS2, *Bacillus cereus* XDS3	Crude oil	4.80– 6.90	She, *et al.*, 2011
B, licheniformis BNP29	Crude oil	9.30 – 22.10	Yakimov, *et al.*, 1997
Bacillus brevis, Bacillus polymyxa, B, licheniformis	Paraffinic oil	18.00	Almeida, *et al.*, 2004
B, licheniformis AC01	Paraffin	21.70	Dastgheib, *et al.*, 2008

IV. CONCLUSION

The NGSI-A15 isolate (*Acinetobacter* sp.) is a thermo-tolerant bacterium with superb biosurfactants production. NGSI-A15 is a prospective isolate for microbial enhanced oil recovery (MEOR). Biosurfactant produced at 65 °C by this isolate had a remarkable additional oil recovery (AOR).

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