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Effect of Microbial Activities on the Viscosity of Heavy crude in Niger Delta Field

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Abstract

This study focuses on the post-recovery effect of Nigerian heavy crude viscosity in microbial enhanced oil recovery process. The technique involves the injection of exogenous hydrocarbon-degrading microbes with the required nutrients to guarantee the bacteria isolates produce the needed metabolites such as biosurfactants, biopolymer, etc. In this study, thirty- seven hydrocarbon-degrading bacteria were isolated from a hydrocarbon-contaminated soil samples from Gio, Tai Local Government Area in Ogoniland. Three of the bacterial isolates were selected and screened for biopolymer and biosurfactant production using Sudan black solution, oil spreading test, emulsification index, and haemolytic assay. The selected microbes were identified as Bacillus sp, Klebsiella sp & Pseudomonas sp by biochemical analysis. The selected microbes were exposed to ranges of salinity, pH, temperature, nutrient sources, and inoculum concentration to determine their optimum performance in reservoir conditions. The result showed the optimum parameter range for the three microbes: pH 7- 8, a temperature within 25 - 400C and salinity within 0.5% - 5%. The best nitrogen source was peptone while the best carbon source for Bacillus sp was glucose and glycerol for Pseudomonas sp and Klebsiella sp respectively. These optimum parameters were employed in the formulation of the nutrient broth used for the core flooding experiment. The core flooding setup was locally fabricated and calibrated. Fourteen microbial samples of pure and consortium were injected with carbon and nitrogen sources. The result showed an additional recovery range between 18.33% to 29.09% of the pore volume. The post-recovery analysis showed drastic transformation (reduction in viscosity) of the heavy crude to light hydrocarbon components by an average of 20.33% with glucose and 97.27% with peptone.

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

Introduction

Goodarzi *et al*. (2009) defined heavy oil in terms of viscosity as oil with viscosity ranging from 50 cP to 5000 cP. At reservoir temperature and pressure, the flow of fluid is restricted by high viscosity. Ruixia and Anhuai (2009) defines heavy oil has a high viscous and dense fluid contains more resins and asphaltenes component.

Ancheyta and Speight (2007) define heavy oil as a crude with high viscosity, which also contains a higher level of sulfur as compared to conventional petroleum. Meyer *et al*., (2007) explained that the crude oil tends to be heavy due to the obliteration of light fractions via natural processes after the formation from sedimentation"the natural source materials".

Petroleum Institute (API) gravity is used to classify oils as: light, medium, heavy, or extra heavy. The API gravity is an important parameter that determines the market value of oil. According to the API manual and the North American taxation system (Trevisan et al., 2006), crude are classified as follows:

A. Light - API > 31.1
B. Medium - API between 22.3 and 31.1
C. Heavy - API > 10 to < 22.3
D. Extra Heavy - API < 10.0

Meyer et al (2007) highlighted some possible processes that favours the formation of naturally occurring bitumen resources and heavy oil in geological basins around the world. In the first instance, they were of the opinion that oil may be forced out from the source rock as immature oil. According to Larter et al (2006), immature oil account for a little fraction of the heavy crude. Secondly, prior to the generation of heavy oil in the trap, it was assumed to be light or medium oil in the source rocks and upon migration to a trapping system, it can be transformed into heavy oil by several processes such as bacterial degradation, water washing, evaporation etc and if there is an elevation of the trap into an oxidizing region. Thirdly, it was proposed that at depth in subsurface formations, biodegradation can occur (Head *et al*, 2003; Larter *et al*., 2006).

Therefore, recovery of heavy crude is usually a challenge due to the viscous nature of the crude. Since the fluid is viscous, injection of water alone is not efficient to recover the oil due to poor sweep efficiency and thus requires large amount of polymer. Hence, in this study, microorganisms that are capable of producing biopolymers, biosurfactants, acids, solvents, gases and biomass will be used as the enhancer instead of injecting these components separately.

Application of Microbes in Enhancing Oil Recovery (MEOR)

Mcroorganisms (microbes) can be injected into oil reservoir in two ways - indigenous and exogenous. The indigenous technique involves the injection of nutrient into oil reservoirs to activite the inherent microbes, thereby enabling them to produce the required metabolites. While exogenuous technique involves the injection of microorganisms with appropriate nutrients into oil reservoirs to help recover the oil beyond the primary and secondary stage as shown in Figure 1.

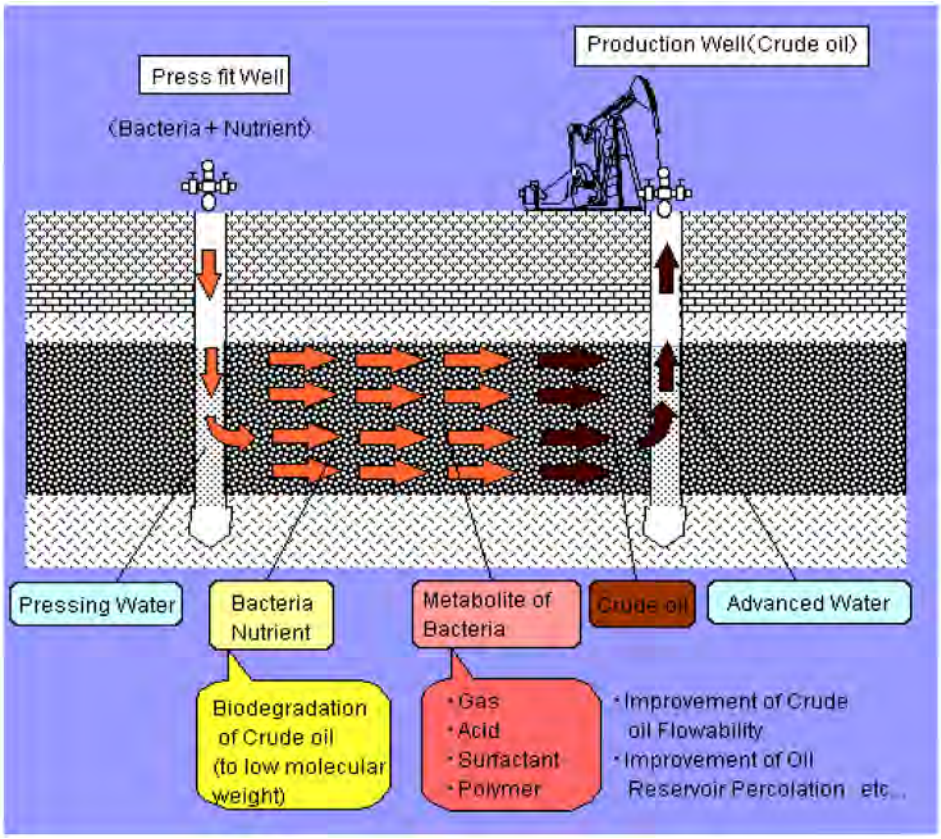

Figure 1—Microbial injection (source: petroblogger.com)

Furthermore, the injected microbes alongside with their nutrient broth grow at exponential rates with time, thereby making it possible to produce enormous amounts of useful products and thus improve the activities of the microbes in the reservoir, while in EOR technologies, the efficiency of the additives such as polymers, alkaline, surfactants tend to decrease with time and distance from the injection well. Hence, the challenge with MEOR techniques is the selection and culturing of the right microorganisms that will decompose the heavier ends of hydrocarbon, withstand or survive to an extent, the reservoir conditions such as high temperature and high pressure (HTHP), high salinity, pH, etc. therefore, the aim of this study is to isolate and characterized the right microorganisms that will produce the required metabolites to effectively reduce the heavy oil viscosity.

Materials and Methods

Materials required

- Soil samples
- Core samples
- Heavy crude sample
- Distilled water
- Mineral salt media (MSM)
- Aluminium foil
- Syringe

- Methylated spirit
- Toluene
- Industrial Salt (sodium chloride)
- Clinical cloves

Equipment Used

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

Methods

Samplying and Processing of Hydrocarbon Contaminated Soil Samples

Hydrocarbon-contaminated soil samples were collected from Gio, Tai Local Government Area (LGA) in Ogoniland, Rivers State. These samples were transferred into a polythene bag and transported to the laboratory via ice pack for physicochemical and microbiological analyses. The samples were collected from five different points between the depths of 0 to 15 cm. The method described by Suja *et al*. (2014) was adopted for processing the soil sample.

Serial dilution

To perform ten-fold serial dilution, 9 mL of normal saline (0.85% NaCl w/v in distilled water) was first distributed into clean 25 mL (28x85 mm) McCartney bottles (Hurst, Australia). The bottles were corked properly, sterilized in an autoclave at 121 °C (15 psi) for 15 min and allowe9*d to cool at room temperature. One gramme of each of the processed soil samples was added to 10 mL of sterile normal saline to make stock solution. From this stock solution, 1 mL was pipetted into a corresponding McCartney bottle containing 9 mL sterile normal saline to make 10^{-1} and thereafter 10^{-2}, 10^{-3}, 10^{-4}, $10^{-5}$and 10^{-6} dilutions.

Isolation of hydrocarbon-degrading bacteria by enrichment method

The processed soil samples were used for the isolation of hydrocarbon-degrading bacteria and comprised seven soil samples obtained from four different locations. The method described by Mittal and Singh (2009) with slight modification was used for test. In brief, 10 g of the processed soil was dissolved in 250 mL Erlenmeyer's flask, containing 100 mL of sterilized normal saline. The flask was votexed at maximum speed

for 2 min and the suspension allowed settling for 5 min. A volume of 5 mL of the supernatant was used to inoculate another separate flask containing 100 mL Bushnell Haas Broth (BHB) (containing in g/L: 0.2 MgSO4.7H20; 0.02 CaCl2.2H2O; 1 KH2PO4; 1 K2HPO4; 1 NH4NO3; 0.05 FeCl3; nystatin- 0.1g; and pH 7.0) supplemented with crude oil (1%, v/v) as the sole carbon and energy source. Tween 80 (0.05% v/v) was added to the broth to enhance hydrocarbon degradation. The procedure was repeated for each of the soil samples and the set-up performed in duplicate. The flasks were incubated in a rotary shaker incubator at 150 rpm for 7 days. At the end of 7 days, successive sub-culturing were done by transferring 5 mL of BHB culture into fresh BHB medium supplemented with crude oil (1%, v/v). The sub-culturing ensured isolation of only oil-tolerant and oil-degrading bacteria.

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

Screening of the Microbes for Biosurfactant and Biopolymer

The method described by Mnif *et al*. (2009) was employed in the isolation of the hydrocarbon degrading bacteria. The isolated bacteria shall be screened for biosurfactant production using the following methods:

i. Emulsification stability test (E24) as described by Ijeoma *et al*. (2016)
ii. Oil displacement techniques as described by Ijeoma *et al*. (2016)
iii. Tilted glass slide test as described by Satpute et al (2008)
iv. Haemolytic assay as described by Chamanrokh *et al* (2008)

The microbes were screened for biobolymer production for 2 days at 37 °C in yeast mold (YM) agar to test for their ability to produce biopolymer. The isolates were characterized as biopolymer positive (+ve) or biopolymer negative (-ve) based on the Sudan Black staining of the bacterial cells. Here the biopolymer positive (+ve) cells picked up the Sudan black stain and appeared dark (blue-black inclusions within the cells) when observed under the light microscope, whereas negative cells were stained by the counter strain (safranin) only and they appeared pink.

Preparation and Upscaling of the Mineral Salt Agar for Flooding

The media prepared for the core flooding experiment to inoculate the different single culture microbes and consortium of microbes containing the following composition presented in Table 1:

Table 1—Mineral salt agar for core flooding

Compound	Weight (g)
$MgSO_4$	0.42
KCl	0.29
KH_2PO_4	0.83
K_2HPO_4	1.25
NH_4NO_3	0.42
NaCl	10
Agar	15

The result of the optimization test show that glucose is the best carbon source and peptone is the best nitrogen source. The prepared mineral medium was split into fourteen 500 ml capacity conical flask and autoclave at 121°C for 15 mins at 1.5 psi. The single and consortium microbes were inoculated into each of the Erlenmeyer flasks and incubated at 30°C for 24 h to allow for multiplication of the microbes.

Results and Discussion

Identification of Bacteria Isolate

This was done following the method of biochemical identification in accordance with Cheesbrough (2004), using morphological characteristics and biochemical tests such as: Gram Stain, Motility Test, Catalase Test, Oxidase Test and Indole Test as presented in Table 2

Table 2—Identification of bacteria isolates

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

Effect of Physicochemical and Nutrient Parameters on Biosurfactant Production

The optimum values obtained during the optimization study were employed in the formulation of the nutrient broth and core flooding experiment. The optimal pH range that favours the growth and production of biopolymer of *Bacillus* sp and *Pseudomonas* sp is 7 - 8, while 6 - 7 for *Klebsiella* sp as shown in Table 3. Therefore, the optimum pH value for *Bacillus* sp and *Klebsiella* sp is 7 while *Pseudomonas* sp is 8. The pH growth range corresponds to the result of Ruixia and Anhuai (2009). The result indicate a low growth and production of biopolymer and biosurfactant at lower and higher pH value with the three microbes. The effect of different incubation temperatures at 25, 30, 35, 40, 45 and 50°C on growth, biosurfactant and biopolymer production after optimal pH had been determined. The bacteria were inoculated in a mineral salt media supplemented with kerosene at the optimum pH for 3 days. The result in Table 3 shows the optimum range and value of temperature, salinity, nitrogen and carbon source. These results do not imply that the selected microbes cannot grow and replicate the required metabolites at values greater than the optimum shown in Table 3. It only indicate the optimum or best range of the parameters.

Table 3—Summary of the optimization results for the three microbes

Microbes	Parameter	Optimum Range	Optimum Value
bacillus sp	pH	7 - 8	7
	Temperature	25 - 35°C	30°C
	Salinity	0.5% - 5%	0.5%
	Nitrogen Source	–	Peptone
	Carbon Source	–	Glucose
Pseudomonas sp	pH	7 - 8	8
	Temperature	25 - 40°C	30°C
	Salinity	0.5% - 9%	0.5%
	Nitrogen Source	–	Peptone
	Carbon Source	–	Glycerol
Klebsiella sp	pH	6 - 7	7

Microbes	Parameter	Optimum Range	Optimum Value
	Temperature	25 - 35°C	30°C
	Salinity	0.5% - 5%	0.5%
	Nitrogen Source	–	Peptone
	Carbon Source	–	Glycerol

Result of Core Flooding Experiment

In this study, both primary and secondary recovery was achieved by injection of water into the core system since we cannot mimic the primary oil recovery, which uses the inherent energy of the reservoir. The tertiary recovery comprises fourteen experimental runs of single and consortium of the three selected microbes. The microbes were injected along with their optimum nutrient broth in Table 3. Figure 3 shows the result of MEOR technique with both carbon and nitrogen sources. Hence, a maximum recovery of 27.13% of the pore volume with the consortium of *Pseudomonas* sp, *Bacillus* sp, & *Klebsiella* sp with glucose as carbon source was obtained. Also, a maximum recovery of 24.81% of the pore volume with peptone as a nitrogen source as shown in Figure 3. The least result for both nitrogen and carbon sources was a pure culture of *Pseudomonas* sp of 17.05% and 15.50% respectively. This results compare with Tingshan *et al*. (2005) result of a maximum recovery of 35.7% in Qinghai heavy oil field and Abdullah *et al* (2015) in the Omani oil field which gave an addition of 16% recovery of the initial oil in place from MEOR process.

Figure 2—Core flooding experimental setup

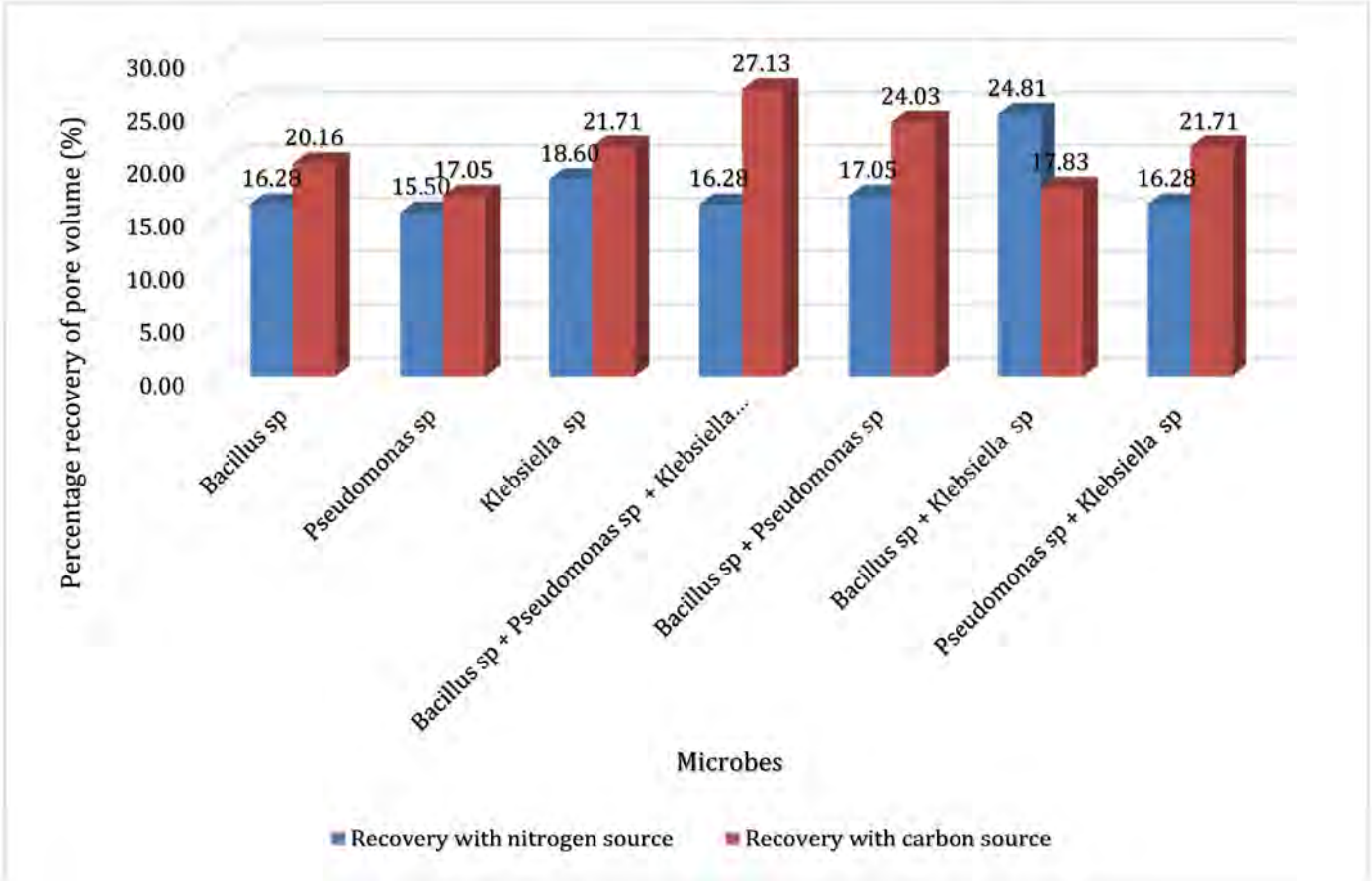

Figure 3—Percentage of pore volume of oil recovered after MEOR technique

Experimental design to check for alteration in the reservoir properties

This experiment was performed to ascertain the effect of the isolated microbes' activity on the recovered heavy crude. The major fluid property considered is the viscosity. To achieve this, the following steps were taken:

i. 600ml of MSM using the composition in Table 1
ii. The sample was split into six 250ml flash as presented in Figure 4
iii. Glucose was added as carbon source into three of the flasks while peptone was added as nitrogen source into the other three flasks
iv. The six samples were incubated with autoclave at 121°C for 15 mins
v. The respective organisms (*Bacillus* sp, *Pseudomonas* sp & *Klebsiella* sp) were inoculated into the six flasks as shown in Figure 4.
vi. The samples were allowed to stay for 24 h for multiplication and production of required metabolites.
vii. 20 ml of microbial nutrient broths were prepared
viii. Finally, 20 ml of the microbial nutrient broths were injected into 50 ml of heavy crude to simulate the post recovered crude with the MEOR technique as presented in Figure 5.

Figure 4—Preparation of MSM

Figure 5—Samples for post recovery analysis

Result of the Effect of Microbial Activities on the Heavy Crude viscosity

A post-recovery analysis was done to evaluate the effect of the microbial activities on the heavy oil viscosity. The viscosity was measured using rheometer R200 rotational viscometer at 500 rpm for 30 seconds. The result of the extra recovery shows large biotransformation of the heavy oil. This implies that most of the heavy oil higher carbon fractions were transformed into lighter hydrocarbon components. The original viscosity (control) of the crude is 208 m.Pa.s and after inoculation of the microbes with glucose and peptone as nutrient sources, the result obtained is presented in Figure 6. *Bacillus* sp reduced the viscosity by 9.62% which is the least among all the pure and consortium of microbes while *Klebsiella* sp yield the best result with 98.17% reduction of the heavy oil with peptone. Comparing the result of the pure bacteria strain with glucose, *Pseudomonas* sp gave the best result of 25.48% and followed by *klebsiella* sp. The result with peptone nutrient gave 98.17% reduction by *Klebsiella* sp, followed by *Bacillus* sp with 97.74 and *Pseudomonas* as least with 96.16% reduction. The result of the consortium of microbes with glucose gave *Bacillus* sp plus *Klebsiella* sp as the highest with reduction by 32.69% and *Klebsiella* sp plus *Pseudomonas*

sp with 97.93% reduction. This result is in agreement with the result obtained by Ruixia and Anhuai (2009) on the transformation of heavy oil in East China of Shengli oil field with the halophilic bacterial strain at a temperature of about 37°C and optimal pH range between 6.5 - 7.0. The result showed a reduction in paraffin contents of the three heavy oils used for the experiments by 4.06%, 28.08% and 55.05%. Therefore, based on the result in Figure 6, all experimental runs with peptone nutrient (nitrogen source) yielded by far better result than glucose nutrient (carbon source).

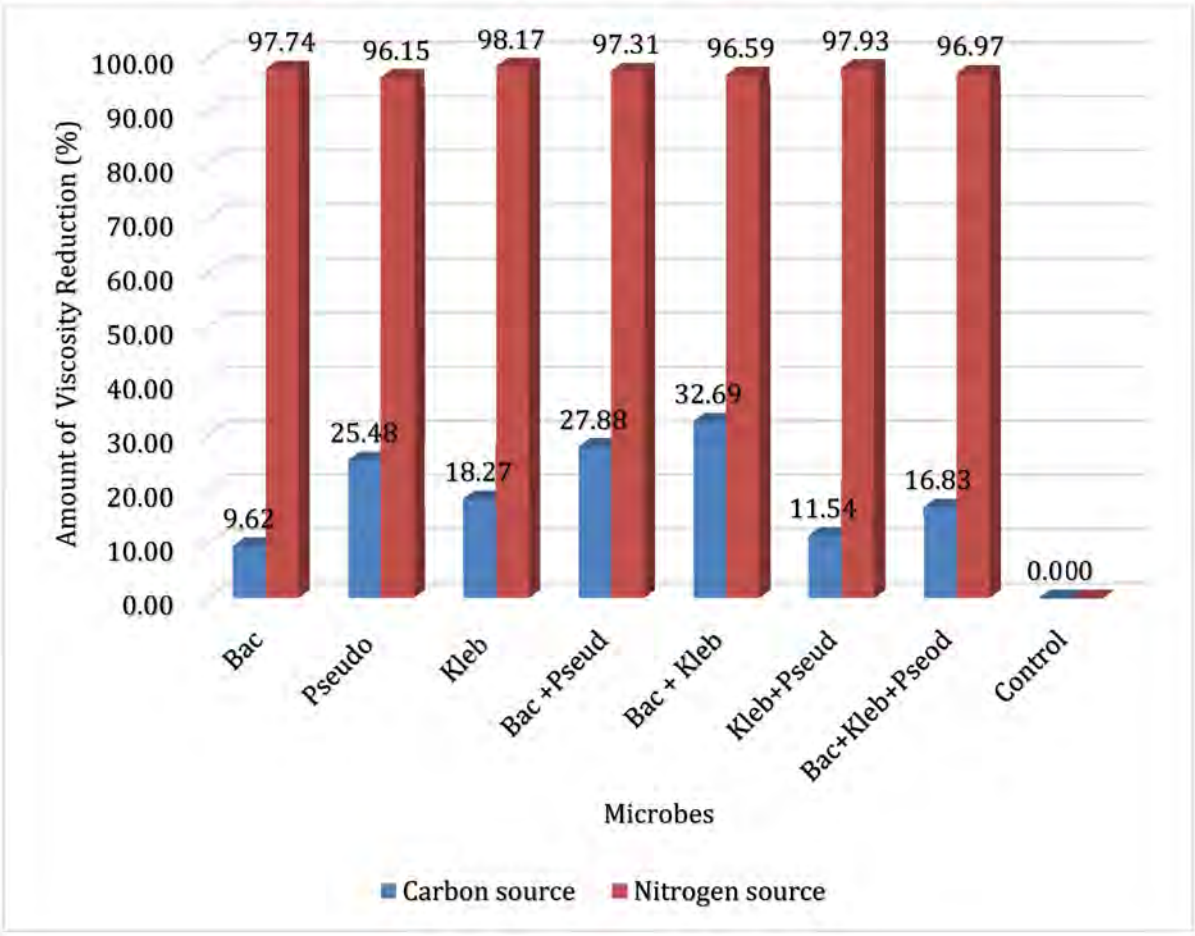

Figure 6—Effect of microbial activities on heavy oil viscosity

Conclusion

i. The result shows that these bacteria can produce biopolymers and biosurfactants that can be injected into oil reservoirs for recovery of the residual oil. Therefore, the growth and production of biosurfactant and biopolymer of the isolated microbes in this study are affected by salinity, temperature, pH, nutrient sources and inoculum concentration.

ii. The MEOR result produced an additional oil recovery of 24.5% with glucose broth and 21.9% with peptone broth. The result of the post recovery analysis showed drastic transformation of the heavy crude to light hydrocarbon components by an average of 20.33% with glucose and 97.27% with peptone.

iii. The injected microbes alongside with their nutrient broth grow at exponential rates with time, thereby making it possible to produce enormous amounts of useful products and thus improve the activities of the microbes in the reservoir, while in EOR technologies, the efficiency of the additives

such as polymers, alkaline, surfactants tend to decrease with time and distance from the injection well.

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