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Laboratory Investigation of Effect of Nanoparticles on the Production of Biosurfacant during Microbial Enhanced Oil Recovery Techniques

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Abstract: *The need for petroleum products in the world is increasing daily, this products can only be gotten from the refining of crude oil a naturally occurring mineral resource which is produced by degradation of organic materials. This crude is gotten from several wells drilled for the sole purpose of the production of this mineral resource but this wells are been shut-down and abandoned because of the decrease in the well's productivity which causes losses to the operators and poor finances for the petroleum industry. Therefore, the need to increase the recovery of a well is been considered, these methods are called enhanced oil recovery method. These methods include Gas injection method, Steam Injection method, Chemical injection method (chemicals such as surfactants, polymers etc) Microbial method and the use of nanoparticles as additives to help increase the recovery process. The type of enhanced oil recovery used in this work is the microbial enhanced oil recovery , this method makes use of biosurfactants which can also be referred to as microbe surfactants. The biosurfactants are gotten from microorganism they act as surfactants which reduces the interfacial tension between the molecules of the substance and the interface, this biosurfactants can be used for many industrial purposes which includes bioremediation, oil recovery etc. Nanoparticles are also another aspect of the recent technology which can increase the recovery of our well providing more crude and finance for the petroleum industry. This work shows how biosurfactants and nanoparticles when used together can increase oil recovery*

Keywords — Micro-organism, Biosurfactants, Nano-particle and Microbial Enhanced Oil Recovery.

1. INTRODUCTION

The demand for energy resources continues to increase with time. Developments in renewable energy are expected to provide sustainable energy and environmentally friendly industries. However, many projects that are related to renewable energy face challenges, including technical, social, and economic challenges. Existing energy resources should optimize production, while avoiding critical environmental risks. Microbial-enhanced oil recovery (MEOR) is an alternative approach to optimize oil production from existing reservoirs. This method is considerably more economic and environmentally friendly than other EOR methods. Energy that is used in microbial processes to enhance oil recovery does not depend on the price of crude oil. Microbes can growth independently under many conditions and produce large amounts of useful products rapidly from cheap, renewable materials that are available in large quantities. As a biological agent, microbial bioproducts are often biodegradable, which results in lower levels of pollution and a low toxicity (Youssef *et al.*, 2009; Khire 2010). MEOR uses microbial activities and various bioproducts to help release residual oil that is trapped inside the rock pores and to stimulate oil flow to the production wells (Safdel *et al.*, 2017). Microorganisms produce biosurfactants, and the latter are important in the MEOR mechanism. Biosurfactants act as surface-active molecules that reduce the interfacial tension (IFT) between different fluid components, and enhance pseudosolubilization of oil in water by creating smaller oil droplets (Khire 2010). Injection of partially purified biosurfactants has increased the amount of recovered oil to 40% surfactants, biosurfactants are biodegradable, nontoxic, characteristically diverse, and stable under extreme conditions. Biosurfactant production can be significantly more affordable, as it may be produced using biomass waste (Gautam and Tyagi 2008; Jing *et al.*, 2011; Dhasayan *et al.*, 2014).The study began with isolation, screening and identification of potential biosurfactant producing bacteria from crude oil sample in reservoir. The biosurfactant from the selected strain was then characterized for its chemical structure. The interactive effect of pH, temperature and salinity on biosurfactant stability was tested by using RSM with the Box–Behnken experimental design. The ability of the biosurfactant to improve oil recovery in the EOR process was also carried out in the study using a core flooding experiment. It was found that the biosurfactant was able to recover about 5.4% of the crude oil from a sandstone core during a flooding experiment. However, further experimentation is necessary to increase the efficacy of biosurfactant activity on enhanced oil recovery, especially related to its interaction with the rock reservoir.

2. METHODOLOGY

2.1 Producion of Biosurfacants

Production of biosurfacant started from collection of contaminated soil and soil is analyzed by the various methods;

i. Serial dilution

ii. Inoculation and Incubation

iii. Enumeration and Isolation of Pure culture

iv. Total Heterotropic Bacteria (THB).

After incubation, pure isolates were obtained by picking distinct culturally and morphologically different colonies from the various plates. These were subjected to streaking on sterile nutrient agar in plates until pure distinct colonies were formed. Biosurfacant were identified and characterized after pure isolates had undergo various biochemical test such as:

i. oxidase test,

ii. Catalase test,

iii. Indole test,

iv. methyl red test,

v. Voges Proskauer test,

vi. Starch hydrolysis test,

vii. Urease test,

viii. Citrate test,

ix. Sugars fermentation test

x. Triple sugar iron agar test

Oil spread diameter, drop collapse activity and emulsification capacity of the culture broth were determined. This was also carried out for a positive (T-POL, a commercial available surfactant) and negative control (Un-inoculated optimized broth and distilled water).
The extraction of the biosurfacant was done by measuring the viscosity and pH of the broth using Hanna multiple pH meter, Cells were the removed from the broth cultures by centrifugation at 5000 rpm for 18 minutes. An L-600 centrifuge was used to achieve this. The liquid supernatant was collected and the sediment materials (cell pellets) discarded.

2.2 Determination of the crude oil properties

The properties of the crude oil used in this work is first determined by a number of experiment, in order for us to know the kind of crude used, the properties test carried out includes

i. Viscosity test was carried with use of redwood viscometer

ii. Density test was carried out using the pyncometer and weighing balance

iii. Flash point test was carried out using Pensky-Martens flash point tester

iv. Cloud point test was carried out using of the ice bath

After the properties test was done the Microbial enhanced oil recovery test was done using:

The set up used for this is a laboratory set up for enhanced oil recovery, this set up can be used various type of enhanced method with includes water, air etc but in this case biosurfactants is used to enhanced the recovery of the crude. The set up is made up of a series of equipment that represents the set up of a well head, the set up makes use of a carbon dioxide cylinder (co_2) that acts as the reservoir pressure, this cylinder is connected to a 12liter metal tank which stands are the reservoir, a pipe is connected to this tank which along its line comprises of a tap handles (stands a the well head valve) and a pressure gauge used to read the tank outlet pressure (reservoir outlet pressure), a condenser to condense any gas if present then a second tap handle which stands as a valve which leads to the collection container (storage tank).
The properties test and microbial enhanced oil recovery test was done for five various sample:

3. RESULT AND DISCUSION

3.1 RESULT OF THE BIOSURFACANT TEST

Different biosurfactants were produced after the sample collection, isolation, incubation, purification and extraction of the microbes. This biosurfactants are gotten from the contaminated soil sample, they are group of bacteria which lives on crude oil contaminated soil so they can also help enhance oil recovery because they feed on the oil causing the crude oil properties to change, In these work only two of the biosurfacant will be used to enhance the recovery of oil. This biosurfactants were cultured and produced in the Microbiology department laboratory and transferred to the petroleum engineering department of the Rivers State University where they are been used for MEOR.

Table 3.1: Cultural and Morphological Characterization of Bacterial Isolates

Key: HUB= Hydrocarbon Utilizing Bacteria

ISOLATES CODES	Colonial Description	Probable Organism
HUB 1	Small, Circular, smooth, convex, opaque and golden yellow colonies with entire margin.	*Staphylococcus sp*
HUB 2	Large, opaque, flat and greenish colonies with irregular margins and distinctively fruity odour colonies	*Pseudomonas* sp
HUB3	Small, smooth, pinkish colonies and round with entire margin.	*Serratia* sp
HUB4	Small, Circular, smooth, convex, opaque and yellow colonies with entire margin.	*Micrococcus* sp
HUB 5	Whitish, slightly convex with irregular edges and opaque colonies	*Bacillus* sp

biosurfacants used for these experiment are:

- HUB 3- *Serratia* sp
- HUB 5- *Bacillus* sp

Key: **HUB** =Hydrocarbon Utilizing Bacteria, **CAT**= catalase, **OXI**= Oxidase, **CIT** = Citrate, **MOT**= Motility, **MR**= Methyl red, **VP** = Voges – Proskauer, IND = Indole, LAC=Lactose **GLU**= Glucose, **SUC** = Sucrose, **MAN**= Manitol.

ISOLATE CODES	GRAM REACTION	SHAPE	CAT	OXI	CIT	MOT	MR	VP	IND	LAC	GLU	SUC	MAN	PROBABLE ORGANISM
HUB 1	+Ve	Cocci	+	+	+	-	+	+	-	A	-	-	-	*Staphylococcus sp*
HUB 2	-Ve	Rod	+	+	-	+	-	+	-	-	-	-	-	*Pseudomonas sp*
HUB3	-Ve	Rods	-	-	-	+	+	+	+	-	A	A	A	*Serratia sp*
HUB4	+Ve	Cocci	+	+	-	+	+	+	-	A	-	-	A	*Micrococcus sp*
HUB 5	+Ve	Rods	+	-	+	+	+	+	-	-	A	-	A	*Bacillus* sp.

3.2 Microbial Enhanced Oil recover Result

The crude oil used were first tested to know the kind of crude used, the properties result shown below:

For Density →

Density=$\frac{\text{weight of filled pycnometer} - \text{weight of empty pycnomete}}{\text{volume of pycnometer}}$ (1)

Empty Pycnometer = 25.2g
Filled Pycnometer = 70.64g
Volume of Pycnometer = 50ml

The density of the crude = $\frac{70.64-25.2}{50}$ = 0.9088g/ml

The specific gravity (S.G) = $\frac{\text{Density of Oil}}{\text{Density of Water}}$ **(2)**

Note: density of water = 1000kg/m^3 or 1g/cm^3

S.G = $\frac{0.9088}{1}$ = 0.9088

API gravity = $\frac{141.5}{\text{S.G}} - 131.5$

= $\frac{141.5}{0.9088} - 131.5$ = 25.20

For Viscosity →

Viscosity $(_{\varphi}) = \left(\text{At} - \frac{\text{B}}{\text{t}}\right)\rho$ **(3)**

Where A = 0.026, B = 0.188, T = time and *P* = density.

Table 4.3 Intial Crude Oil Viscosity at Different Temperature.

Temperature	Time (sec)	Viscosity
30^{o}c	4.24	0.0787
60^{o}c	3.95	0.0501
90^{o}c	3.70	0.4125

Table 3.3 Intial Crude Oil Viscosity at Different Temperature.

Properties	Values
Density @ 30^{o}c	0.9088
API Gravity	25.20
Viscosity @ 30^{o}c	0.0787

Flash point = 104^{o}c **Cloud point =** 2.8^{o}c

Table 3.4 Properties of crude oil

Having done the properties test of the crude oil, it was known that the crude used is a LIGHT CRUDE.

Samples	Inlet Pressure (Bar)	Outlet Pressure (Bar)	Time (Sec)	Volume Recovered (Liter)

Sample A	3.5	1.7	10	8.8
Sample B	3.5	1.4	10	7
Sample C	3.5	1.5	10	9.2
Sample D	3.5	1.5	10	7
Sample E	3.5	1.7	10	9.2

Table 3.5 Oil Recovery Result.

Note that : Sample A = 4liters of crude oil & 6liters of Water
Sample B = 4liters of crude oil, 6liters of water & 0.4liters of Bacillus
Sample C = 4liters of crude oil, 6liters of water & 0.4liters of Serratia

= 3.15liters ≈ 3.2liters

Table 3.:6 Total Oil Recovered

Sample	**Total Volume Recovered**	**Water cut**	**Oil Recovered**
Sample A	8.8	5.8	3.0
Sample B	7	3.8	3.2
Sample C	9.2	5.5	3.7
Sample D	7	3.5	3.5
Sample E	9.2	5.2	4.0

For Properties Test Result →
After oil was recovered for the following sample, crude oil properties test was carried out for each of them to know which of the properties the biosurfactants and the nanoparticles changed that help us to recover more crude.

SAMPLE A

Filled pycnometer = 71.0g Empty Pyncnometer =25.2g Volume of Pycnometer = 50ml

$$\text{Density} = \frac{\text{filled pycnometer} - \text{empty pycnometer}}{\text{volume of pycnometer}}$$

$$\text{Density} = \frac{71-25.2}{50} = 0.916\text{g/ml}$$

$$\text{The specific gravity (S.G)} = \frac{\text{Density of Oil}}{\text{Density of Water}}$$

$$\text{S.G} = \frac{0.916}{1} = 0.916$$

$$\text{API gravity} = \frac{141.5}{\text{S.G}} - 131.5$$

$$= \frac{141.5}{0.916} - 131.5 = 22.97$$

For Viscosity →

$$\text{Viscosity } (_{\varphi}) = \left(\text{At} - \frac{\text{B}}{\text{t}}\right)\rho$$

Where A = 0.026, B = 0.188, T = time and *P* = density

Table 3.7 Table of Sample A Viscosity

Flash point = 68^0c

SAMPLE B

Filled pycnometer = 71.23g Empty Pyncnometer =25.17g Volume of Pycnometer = 50ml

$$\text{Density} = \frac{\text{filled pycnometer} - \text{empty pycnometer}}{\text{volume of pycnometer}}$$

$$\text{Density} = \frac{71.23-25.17}{50} = 0.9212\text{g/ml}$$

$$\text{The specific gravity (S.G)} = \frac{\text{Density of Oil}}{\text{Density of Water}}$$

$$\text{S.G} = \frac{0.9212}{1} = 0.9212$$

$$\text{API gravity} = \frac{141.5}{\text{S.G}} - 131.5$$

$$= \frac{141.5}{0.9212} - 131.5 = 22.10$$

For Viscosity →

$$\text{Viscosity } (_{\varphi}) = \left(\text{At} - \frac{\text{B}}{\text{t}}\right)\rho$$

Where A = 0.026, B = 0.188, T = time and *P* = density

FOR SAMPLE C

Filled pycnometer = 70.98g Empty Pyncnometer =25.16g Volume of Pycnometer = 50ml

$$\text{Density} = \frac{\text{filled pycnometer} - \text{empty pycnometer}}{\text{volume of pycnometer}}$$

$$\text{Density} = \frac{70.98-25.16}{50} = 0.9164\text{g/ml}$$

The specific gravity (S.G) = $\frac{\text{Density of Oil}}{\text{Density of Water}}$

S.G = $\frac{0.9164}{1}$ = 0.9164

API gravity = $\frac{141.5}{\text{S.G}} - 131.5$

= $\frac{141.5}{0.9164} - 131.5 = 22.91$

For Viscosity →

Viscosity ($_{\varphi}$) = $\left(\text{At} - \frac{\text{B}}{\text{t}}\right)\rho$

Where A = 0.026, B = 0.188, T = time and *P* = density

Flash Point = 68^0c

SAMPLE D

Filled Pycnometer= 71.2g EmptyPycnometer=25.29g
Volume of Pycnometer = 50ml

Density = $\frac{\text{filled pycnometer} - \text{empty pycnometer}}{\text{volume of pycnometer}}$

Density = $\frac{71.2-25.29}{50}$ = 0.9182g/ml

pecific gravity (S.G) = $\frac{\text{Density of Oil}}{\text{Density of Water}}$

S.G = $\frac{0.9182}{1}$ = 0.9182g/ml

API gravity = $\frac{141.5}{\text{S.G}} - 131.5$

= $\frac{141.5}{0.9182} - 131.5 = 22.60^0$

For Viscosity→

Viscosity ($_{\varphi}$) = $\left(\text{At} - \frac{\text{B}}{\text{t}}\right)\rho$

Where A = 0.026, B = 0.188, T = 4.77 and *P* = density

SAMPLE E

FilledPycnometer=71.04gEmptyPycnometer=25.21g
Volume of Pycnometer = 50ml

Density = $\frac{\text{filled pycnometer} - \text{empty pycnometer}}{\text{volume of pycnometer}}$

Density = $\frac{71.04-25.21}{50}$ = 0.9166g/ml

From Equation 4.2, specific gravity (S.G) = $\frac{\text{Density of Oil}}{\text{Density of Water}}$

S.G = $\frac{0.9166}{1}$ = 0.9166g/ml

Recall from Equation 4.3, API gravity = $\frac{141.5}{\text{S.G}} - 131.5$

= $\frac{141.5}{0.9166} - 131.5 = 22.87^0$

Recall Equation 4.4, Viscosity ($_{\varphi}$) = $\left(\text{At} - \frac{\text{B}}{\text{t}}\right)\rho$

Where A = 0.026, B = 0.188, T = 4.24 and *P* = density

Flash Point = 68^0c

RELATIONSHIP BETWEEN TEMPERATURE AND VISCOSITY.

As the temperature of the crude oil increases, the viscosity of the crude decreases. Viscosity as we know is the resistance of a fluid to flow, viscosity is the thickness of a fluid which hinders it from flowing. Therefore if the temperature increase of the crude oil cause a reduction of viscosity, it means as the temperature of the oil increases the ability of the crude oil to flow more increases.

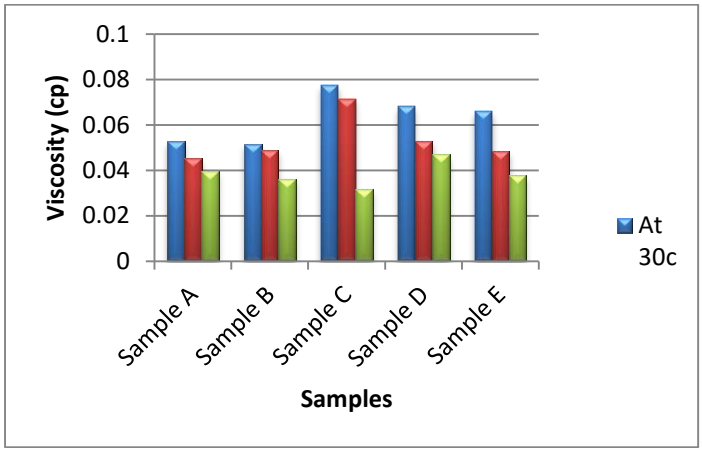

Figure 4.1: Graph Showing The Relationship Between Temperature & Viscosity

From the above chart, at 30^0c the sample with the least viscosity in the ascending order are , Sample B, Sample A, Sample E, Sample D and then Sample C. This means that at the temperature of 30^0c Sample B flows faster than the other sample.

At 60^0c its Sample A, Sample E, Sample B, Sample D and Sample C, which implies that at this temperature the more viscous sample is Sample C and the less viscous sample is Sample A.

At lastly at 90^0c, the most viscous fluids are Sample D, Sample A and D. Sample C and Sample B are less viscous because they took a lesser time to flow under a standard condition.

4.6 RELATIONSHIP BETWEEN SPECIFIC GRAVITY AND API GRAVITY

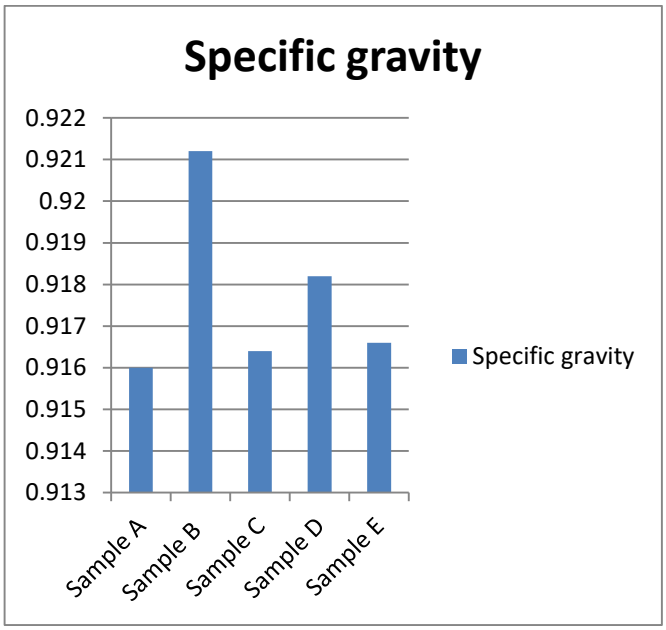

Figure 4.2: Graph of Specific Gravity

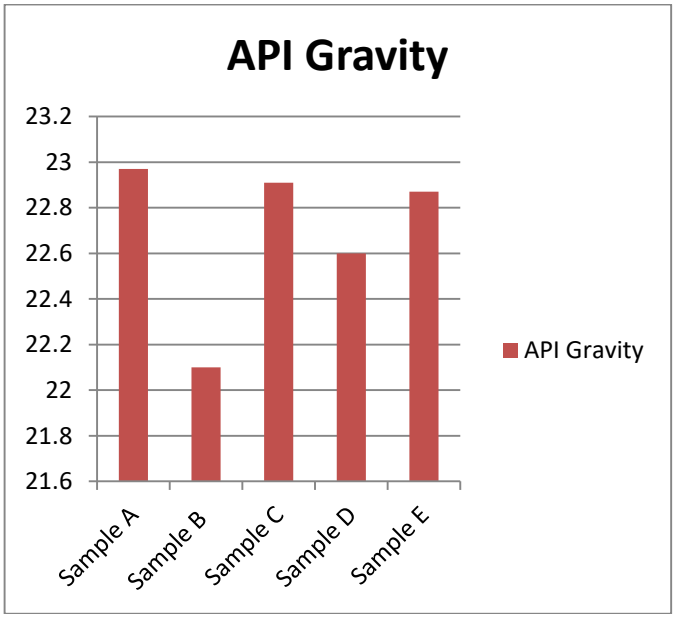

Figure 4.3: Graph Of API Gravity

API gravity it is the measure of how heavy or light a product is in comparison to water. If a products API is less than 10, it is heavy and can sink in water but if it's the opposite, it can float in water while specific gravity has to do with the density of a particular object divided by the density of water, which means it is the comparison between the density of an equal volume of liquid and water at a specific temperature. The relationship is the lower the specific gravity the higher the API gravity.

From the chart above, the samples with the lowest specific gravity are Sample B and Sample D has the higher API gravity which makes them the lighter crude sample.

Samples with the lowers API gravities are Sample A, Sample C and Sample E which has the highest specific gravity which means they are the heaviest crude samples.

4.7 RELATIONSHIP BETWEEN TOTAL VOLUME RECOVERED AND OIL RECOVERED.

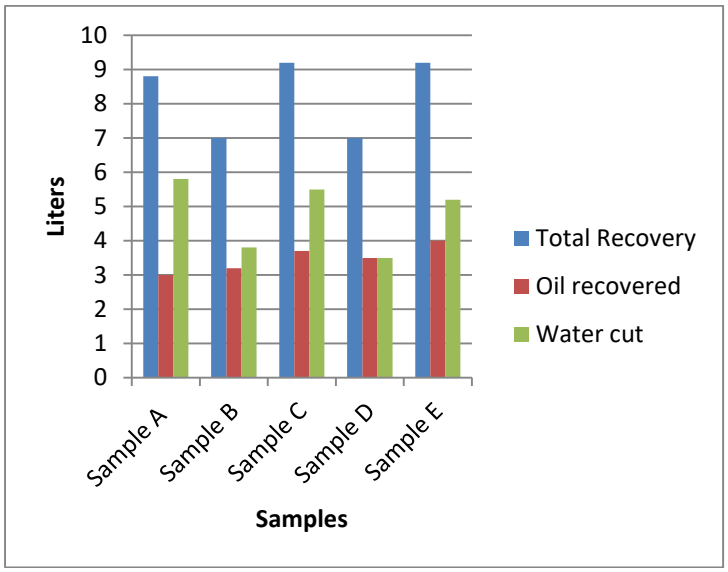

Figure 4.4: A Graph Showing The Total Recovery, Oil Recovered And Water Cut

The Total volume of the recovery for the different samples was gotten under the same temperature and pressure conditions containing samples of the same quantity and properties. The samples total recovery volume varies and so also does the oil recovered for each sample vary.

From the graph above, the samples with the largest volume recovered are sample A, sample C and sample E; sample B and sample D had the lowest volume recovered.

The samples with the highest volume of oil recovered are sample E and sample C, followed by sample D and sample B, sample A had the lowest volume of oil recovered.

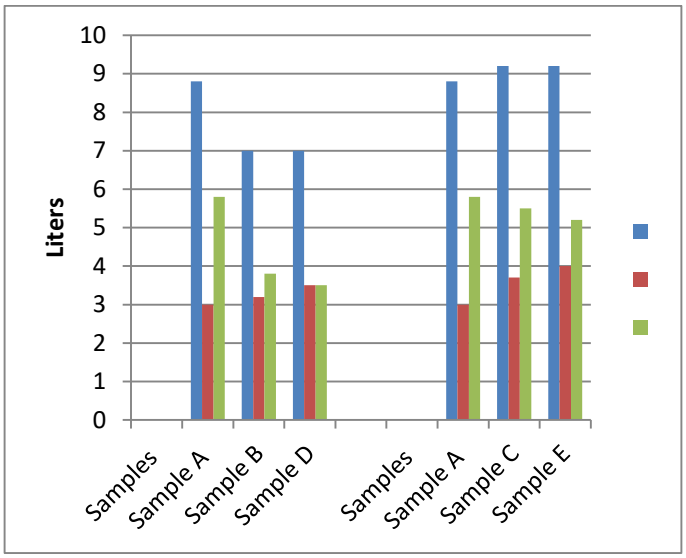

Figure 4.5: A Graph Showing The Recovery Between The Two Biosurfactants Used.

Sample B and D are samples containing Bacillus, they did not recovery a lot but they recovered crude oil slightly above the control sample which is Sample A. Sample C and E are samples containing Serratia, the biosurfactants used recovered more crude than the control sample. Sample D is the sample containing Bacillus and Nanoparticle, the sample did not recover a lot which means the nanoparticles when added to the bacillus causes the Bacillus to degrade which in turn causes the recovery to reduce.

4. CONCLUSION AND RECOMMENDATION

4.1 Conclusion

Factors influencing biosurfactants production are the nature of the carbon source, nitrogen source, temperature, aeration and pH. Ammonium salts and urea are preferred nitrogen sources for biosurfactant production. The above result confirms that biosurfactant are active biomolecules that can be used in oil recovery.

Biosurfactants generally increases the recovery from a reservoir because of its tendency to change the crude oil properties like viscosity and API gravity, The biosurfactant was successfully used in enhanced oil recovery in consolidated laboratory cores.

From the microbial enhanced oil recovery carried out with a laboratory set up, the result is concluded as follows;

i. Sample A which was crude oil and water, had a high volume of recovery but a lower volume of crude oil recovered.
ii. Sample C and E are samples containing crude and the Serratia biosurfactants in them, they also had a high volume of total recovery and also a high volume of crude oil recovery too
iii. Sample B and D are samples containing the crude and Bacillus biosurfactants, their recovery was low compared to other samples but they also recovered more crude than Sample A which was just Crude oil and water alone.
iv. Sample E and C are samples containing Aluminum oxide (nanoparticles), they recovered more crude than the other samples containing the same biosurfactants with them, that means addition of nanoparticles help to recover more crude from the sample
v. Sample E had the highest total recovery and the highest oil recovered which means that Serratia which is contained in it was very active and with the help of the nanoparticles in it, crude oil recovery was more effective.
vi. The interfacial surface tension of the crude oil was reduced when biosurfacant was added

4.2 Recommendation

The objective of every work is to obtain results which are not only practicable but also helps economically. Therefore from this work and practical, the following recommendations are made:

i. The use of biosurfactants in the petroleum industry will be of great help for the recovery of Crude Oil in the industry only if the idea will be put into consideration and practice..
ii. The effect of the biosurfactants should be investigated more to know if there will be any effect on the crude properties and the reservoir as a whole.
iii. In the enhanced oil recovery process, the crude oil temperature should be increased a bit before recovery starts because increase in temperature leads to a reduction of the viscosity of the crude.
iv. More biosurfacant should be tested to know which best enchance the production of crude oil.
v. The use of Nanopraticles, Nanofluids and Nanotechnology is a new advances in the Energy industry as a whole, they will rapidly increase the recovery of oil if put into practice in the industry.

Heavy crude shoulda also be tested with various biosurfacant

4.3 Contribution to knowlegde

Biosurfacants aids in oil recovery and should be used often, It is more economical than other methods of enhance oil recovery and it can be used in various aspect of the industry including the cleaning of oil spillage, cleaning of oil pipelines. A wide reange of study should also be carried out to find out more economic importance of biosurfacant in the oil industry

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