Laboratory Determination of Productivity of Bomu Oil Field (Oml 11 as Case Study) Using MEOR Process and Calcium Oxide Nanoparticles

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Abstract
The need for petroleum products in the world is increasing daily, this product 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 being 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. The ever-rising global demand for energy and the issue of large volumes of unrecovered oil after primary and secondary oil production operations are driving the development and/or advancement of enhanced oil recovery (EOR) techniques. Conventional EOR processes include thermal, immiscible and miscible gas injection, chemical, and microbial enhanced oil recovery (MEOR), among others.

This chapter provides an overview of MEOR including its history, strata microflora, mechanisms of MEOR for oil recovery, and a brief recount of field MEOR applications.

Keywords— *Productivity, Oil Field, MEOR, Calcium Oxide, Bacteria and Nanoparticle*

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I. Introduction

The necessity of improving and advancing the current enhanced oil recovery (EOR) processes to make them more efficient has attracted the attention of researchers and oil field operators. Thus, over the last few decades, this problem has received constant attention resulting in slow but steady growth of the average oil recovery factors. For instance, at present the worldwide average recovery rate is about 30%, whereas in the USA, the average oil recovery factor is 39%. However, many experts believe that in the foreseeable future the recovery factor may well reach 50–60% and even 70–80% [1]

Biosurfactants can be defined as the surface-active biomolecules produced by microorganisms with wide-range of applications. In recent years, due to their unique properties like specificity, low toxicity and relative ease of preparation, these surface-active biomolecules have attracted wide interest. Due to their unique functional properties, biosurfactants were used in several industries including organic chemicals, petroleum, petrochemicals, mining, metallurgy (mainly bioleaching), agrochemicals, fertilizers, foods, beverages, cosmetics, pharmaceuticals and many others. They can be used as emulsifiers as well as demulsifiers, wetting agents, foaming agents, spreading agents, functional food ingredients and detergents. The interfacial surface tension reducing ability of biosurfactants made them to play important role in oil recovery and bioremediation of heavy crude oil (Volkering *et al.,* 1998). The three major functions played by biosurfactants including (Rosenberg and Ron, 1999). They were used to increase the surface area of hydrophobic substrates. Biosurfactants also used to increase the bioavailability of hydrophobic substrates through solubilization/desorption. They also regulate the attachment and removal of microorganisms from the surfaces [2]

When compared to chemical or synthetic surfactants, biosurfactants gained several advantages including their biodegradability, biocompatability and digestibility. The biosurfactants can be used in environmental cleanup by biodegradation and detoxification of industrial effluents and in bioremediation of contaminated soil. Their specificity and availability of raw materials also made them most preferred surfactants [2]

Properties: The unique and distinct properties of biosurfactants when compared to their chemically synthesized counterparts and broad substrate availability made them suitable for commercial applications. The distinctive features of microbial surfactants are related to their surface activity, tolerance to pH, temperature and ionic strength, biodegradability, low toxicity, emulsifying and demulsifying ability and antimicrobial activity [3].

A. *Temperature and pH Tolerance:*

The biosurfactant production from extremophiles has gained attention in last decades for their considered commercial interest. Most of the biosurfactants and their surface activity are resistant towards environmental factors such as temperature and pH. McInerney *et al*., Reported that lichenysin from Bacillus licheniformis was found to be resistant to temperature up to 50°C, pH between 4.5 and 9.0 and NaCl and Ca concentrations up to 50 and 25 g LG1, respectively. Another biosurfactant produced by Arthrobacter protophormiae was found to be both thermostable (30-100°C) and pH (2 to 12) stable [4] Since, industrial processes involve

exposure to extremes of temperature, pH and pressure, it is necessary to isolate novel microbial products that able to function under these conditions

B. *Biodegradability*

Microbial derived compounds can be easily degraded when compared to synthetic surfactants [2] and are suitable for environmental applications such as bioremediation/biosorption [4] The increasing environmental concern forces us to search for alternative products such as biosurfactants. Synthetic chemical surfactants impose environmental problems and hence, biodegradable biosurfactants from marine microorganisms were concerned for the biosorption of poorly soluble polycyclic aromatic hydrocarbon, phenanthrene contaminated in aquatic surfaces. Controlled the blooms of marine algae, Cochlodinium using the biodegradable biosurfactant sophorolipid with the removal efficiency of 90% in 30 min treatment.

C. *Low Toxicity*

Although, very few literatures were available regarding the toxicity of biosurfactants, they are generally considered low or non-toxic products and are appropriate for pharmaceutical, cosmetic and food uses. [5] demonstrated the higher toxicity of the chemical-derived surfactant (Corexit) which displayed a LC50 against Photobacterium phosphoreum and was found to be 10 times lower than of rhamnolipids. [6] compared the toxicity and mutagenicity profile of biosurfactant from Pseudomonas aeruginosa and chemically derived surfactants and indicated the biosurfactant as non-toxic and non-mutagenic. The low toxicity profile of biosurfactant, sophorolipids from Candida bombicola made them useful in food industries. Emulsion forming and emulsion breaking: Biosurfactants may act as emulsifiers or de-emulsifiers. An emulsion can be described as a heterogeneous system, consisting of one immiscible liquid dispersed in another in the form of droplets, whose diameter in general exceeds 0.1 mm. Emulsions are generally two types: oil-in-water (o/w) or water-in-oil (w/o) emulsions. They possess a minimal stability which may be stabilized by additives such as biosurfactants and can be maintained as stable emulsions for months to years. Liposan is a water-soluble emulsifier synthesized by Candida lipolytica which have been used to emulsify edible oils by coating droplets of oil, thus forming stable emulsions. These liposans were commonly used in cosmetics and food industries for making oil/water emulsions for making stable emulsions.

D. *Antisemite Agents*

A biofilm can be described as a group of bacteria/other organic matter that have colonized/accumulated on any surface. The first step on biofilm establishment is bacterial adherence over the surface was affected by various factors including type of microorganism, hydrophobicity and electrical charges of surface, environmental conditions and ability of microorganisms to produce extracellular polymers that help cells to anchor to surfaces. The biosurfactants can be used in altering the hydrophobicity of the surface which in turn affects the adhesion of microbes over the surface. A surfactant from Streptococcus thermophilus slows down the colonization of other thermophilic strains of Streptococcus over the steel which are responsible for fouling. Similarly, a biosurfactant from Pseudomonas fluorescens inhibited the attachment of Listeria monocytogenes onto steel surface [6].

II. LITERATURE REVIEW

A. *Bomu Oilfield (OML 11)*

The Bomu oil field with the WELL NO. OML 11 is an oil field located in Gokana Local Government Area of the Eastern Niger Delta region of Nigeria. The discovery well was spudded in February 1958 and discovered 265 ft of gas sands and 165 feet of oil bearing sands of the Agbada formation. Production began in

1959 and the first stage of a 12-inch pipeline connecting Bomu to the town of Bonny through Anam was completed in the same year.

Prior to the Nigerian Civil War, production reached 75,000 barrels per day with 26 oil producing wells and 3 water producing wells. In 1970, a blow-out occurred in one of the production wells at Bomu leading to the destruction of vegetation and farm crops. The giant Bomu oil field in Ogoni located in Gokana Local Government Area of River State in Nigeria, which has estimated ultimate recovery of 0.311 billion of barrels of oil and a total of 0.608 billion of barrels of oil equivalent including gas, was discovered in 1958. According to Amanyie (2005; 2006), in the late 1950s oil was struck in commercial quantity at Lekuma Khana in Ogoni soil, and in Bomu oil field in 1958. In 1962 and 1963, the Korokoro and Ebubu fields were respectively discovered and by 1964, the oil refinery at Elesa Eleme went in to full production.

B. *Enhanced Oil Recovery (EOR)*

The use of petroleum product increases every day and as such the need for crude oil recovery increases also. After the conventional recovery method has been carried out, about 50-70% of crude oil remains unrecovered and enhanced oil recovery helps in the recovery of such crude. Enhanced oil recovery methods include Gas injection (nitrogen injection, CO_2 injection and hydrocarbon gas injection), chemical injection, Microbial Enhanced oil recovery (MEOR). This various method helps to reduce the fluid viscosity, reduce the surface tension, reduce the interfacial tension between reservoir fluid, increase the reservoir permeability, increase the reservoir wettability, increase mobility ratio and increase sweep efficiency which enhances the production of residual oil from the reservoir.

The development of an oilfield refers to the process of displacing the accumulated liquid and gas hydrocarbons in the reservoir towards production wells. Oil is produced initially using the natural driving energy of the reservoir (primary recovery operations), or by introducing energy into the reservoir during secondary oil recovery (water flooding or gas flooding), as soon as the natural reservoir energy is depleted.

Particularly, in Kazakhstan most of the hydrocarbon deposits have already been discovered and commercially produced. Currently, fewer and fewer drilling sites in those mature reservoirs are of interest from the commercial standpoint. In this regard, the residual oil left behind in these mature hydrocarbon deposits after primary and secondary oil recovery offers an opportunity for the implementation of EOR processes, including the application of microbial enhanced oil recovery (MEOR) technology. This enhanced oil recovery method is done by the production of biosurfactants by microbes, this method is becoming rapid with the use of micro-organism and their metabolic nature helps enhance the recovery of residual oil by. This biosurfactants has surface active molecule which helps in the reduction of interfacial tension between oil and water and formation of micelles. Microbial enhanced oil recovery method has been research since 1960, this organic microbe reacts with carbon source to form a surfactant which in turn enhances oil [7]

The necessity of improving and/or advancing the current enhanced oil recovery (EOR) processes to make them more efficient has attracted the attention of researchers and oil field operators. Thus, over the last few decades, this problem has received constant attention resulting in slow but steady growth of the average oil recovery factors. For instance, at present the worldwide average recovery rate is about 30%, whereas in the USA, the average oil recovery factor is 39%. However, many experts believe that in the foreseeable future the recovery factor may well reach 50–60% and even 70–80% [8].

I. Advantages of MEOR

These are the advantages of Microbial Enhanced oil recovery

1. The injected bacteria and nutrient are inexpensive and easy to obtain and handle in the field.
2. MEOR processes are economically attractive for marginally producing oil fields and are suitable alternatives before the abandonment of marginal wells.
3. Microbial cell factories need little input of energy to produce the MEOR agents.
4. Compared to other EOR technologies, less modification of the existing field characteristics is required to implement the recovery process by MEOR technologies, which are more cost-effective to install and more easily applied.
5. Since the injected fluids are not petrochemicals, their costs are not dependent on the global crude oil price.
6. MEOR processes are particularly suited for carbonate oil reservoirs where some EOR technologies cannot be applied efficiently.
7. The effects of bacterial activity within the reservoir are improved by their growth with time, while in EOR technologies the effects of the additives tend to decrease with time and distance from the injection well.
8. MEOR products are all biodegradable and will not be accumulated in the environment, therefore are environmentally compatible.

II. Disadvantages of MEOR

1. It is a complex process because the desired bacterial activities depend on the physical and chemical characteristics of the reservoir.
2. The majority of MEOR field projects are conducted on stripper wells, which renders MEOR a low incremental oil recovery process.
3. It is a slower process than chemical or thermal EOR, and usually takes weeks or even months before any benefits are observed.
4. MEOR is hard to control once implemented in the field, and its success is difficult to predict due to high heterogeneity of reservoirs.
5. The cultivation of microorganisms in the laboratory that can grow and/or produce the desired metabolic products (e.g.biosurfactants) under reservoir conditions has proven difficult.

During water flooding, dissolved oxygen in the water is delivered to the strata. This oxygen is rapidly consumed at the bottom-hole area during the microbial oxidation of residual oil, which causes the stimulation of anaerobic bacteria, particularly, methanogens, in the oxygen-free zone of the stratum. The number of aerobic hydrocarbon-oxidizing and oligo carbophilic bacteria grows to a less extent, however, at the same time the content of anaerobic metabolism products in oil formation waters increases significantly. During this process, carbonate and sulphate content increases.

Foam-forming organic substances that reduce the interfacial tension at the oil/water interface by a factor of 100 have been identified to be caused by microbiological oil oxidation products in the bottom-hole area.

Oil recovery by water flooding can be viewed as a natural fermenter, where continuous microbial cultures could be maintained. These microbial cultures could be activated by the injection of microorganisms and nutritional medium components. In this case, residual oil serves as the main substrate for microorganisms; while in the bottom-hole area, a specific microbial community is formed whose activity can be regulated [9].

E. Bio-Surfactants-Structure and Types

Biosurfactants are made up of a hydrophilic moiety, which may be an acid, peptide, cations, anions, mono-, di-, or polysachharides; and a hydrophobic moiety, which may be unsaturated,

or saturated hydrocarbon chains, or fatty acids [9]. These compounds are structurally diverse and can be classified mainly by their chemical nature and microbial origin. They are broadly divided into two classeslow molecular weight molecules which include glycolipids, lipopeptides and flavolipids; high molecular weight molecules such as polysaccharides, proteins, lipopolysaccharides and lipoproteins. The low molecular surface active agents are known as biosurfactants and the high molecular weight agents are bioemulsifiers. Established on their chemical nature, biosurfactants can be further split into six classes: glycolipids, lipopolysachharides, lipoproteins-lipopeptides, fatty acids, neutral lipids and phospholipids, polymeric and particulate biosurfactants [9]. Different microbial groups such as yeasts, actinomycetes, filamentous fungi and bacteria have been reported so far as the producers of biosurfactants [9].

A. Bio-surfactants in Petroleum Industries

Microbial enhanced oil recovery: It is a tertiary oil recovery technique, which uses microbes and/or their metabolites to recover remaining oil from reservoir after the primary and secondary recovery procedures. Bio-surfactants produced by microorganisms' aid in microbial enhanced oil recovery (MEOR). Widespread research has been carried out on a laboratory scale on sand pack columns and field trials have also been conducted in this area. All the bacterial strains produced bio-surfactants which was efficient in emulsifying crude oil. Further the bio-surfactants showed a good stability at extreme environmental conditions (pH 4, 25 g/L of salinity and a temperature of 120°C) similar to those found in oil reservoirs. Laboratory scale oil displacement experiment was carried out with kerosene and 25% of residual oil was recovered by the bio-surfactant from Bacillus subtilis. Fermentative production of the bio-surfactant from Bacillus strains was carried out and efficiency of crude bio-surfactant preparation varied from 30.22-34.19% of the water flood residual oil saturation in sand pack column [10]. There have been numerous reports on the ability of bio-surfactants produced by Bacillus strains for MEOR [11] Other studies which reported the efficiency of bio-surfactants in enhanced oil recovery includes, rhamnolipids produced by Pseudomonas aeruginosa recovered 27% of the original oil after water flooding in sand packed column; bio-surfactant produced by Rhodococcus species. strain recovered 70% of residual oil from oil saturated sand packs.

take out the oily sludge deposits that are produced during oil transportation to refineries in large containers (tankers, barges, trucks) as well as during oil production and processing. In a field trial conducted at the Kuwait Oil Company using two tons of rhamnolipid bio-surfactant produced by a culture broth, sludge was efficiently lifted and mobilized from the bottom of the tank and was solubilized within the emulsion. Approximately 91% of the hydrocarbons were recovered from the sludge [12] Since then extensive researches have been carried out and led to the development of the BioRecoil process patented in 2004 by Idrabel Italia and Jeneil Bio-surfactant Company [12].

Oil transportation and pipeline: After extraction from the fields, crude oil often needs to be transported over long distances to reach the refineries. One of the limiting factors of pipelining is high oil viscosity that slows down the oil flow. In a field trial, it was found that emulsan reduced the viscocity of Boscan crude oil from 200000 centipoises to 70 centipoises, which was then pumped at a rate of 380 miles over 64 hours. It was also calculated that under optimum conditions the transportation rate could be raised to 26000 miles [12].

In another study, emulsan produced by Acinetobacter calcoeceticus PTCC 1318 at 25°C, 30 mg/L, with a water-oil ratio of 1:2 showed 98% emulsification of crude oil and also demonstrated tube cleaning with removal percentages of 100% at room temperature, depending on washing conditions (. On the

other hand, waxy crude oil transportation suffers from the problem of paraffin precipitation that may reduce or even block the internal diameters of pipelines and may also cause a change in oil composition. Biosurfactants produced by Gordonia amicalis LH3 had potential in paraffin control. Pseudomonas and Bacillus and a mixed consortium were capable of degrading n-paraffin used in the treatment of two paraffinic oils.

B. Factors Affecting Bio-surfactant Production

The composition and emulsifying activity of the biosurfactant not only depends on the producer strain but also on the culture conditions, thus, the nature of the carbon source, the nitrogen source as well as the ratio, nutritional limitations, chemical and physical parameters such as temperature, aeration, divalent cations and pH influence not only the amount of biosurfactant produced but also the type of polymer produced.

i. Carbon Sources: The quality and quantity of biosurfactant production are affected and influenced by the nature of the carbon substrate. Diesel, crude oil, glucose, sucrose, glycerol has been reported to be a good source of carbon substrate for biosurfactant production.
ii. Nitrogen Sources: Nitrogen is important in the biosurfactant production medium because it is essential for microbial growth as protein and enzyme syntheses depend on it. Different nitrogen compounds have been used for the production of bio-surfactants such as urea peptone, yeast extract, ammonium sulphate, ammonium nitrate, sodium nitrate, meat extract and malt extracts. Though yeast extract is the most used nitrogen source for biosurfactant production, its usage with respect to concentration is organism and culture medium dependent. Ammonium salts and urea are preferred nitrogen sources for biosurfactant production by Arthrobacter paraffineus whereas nitrate supports maximum surfactant production.
iii. Environmental Factors: These are extremely important in the yield and characteristics of the biosurfactant produced. To obtain large quantities of biosurfactants, it is always necessary to optimize the bioprocess as the product may be affected by changes in temperature, pH, aeration or agitation speed. Most biosurfactant productions are reported to be performed in a temperature range of 25-300. The effect of pH on biosurfactant produced was studied by Zinjarde and Pant who reported that the best production occurred when the pH was 8.0 which is the natural pH of sea water.
iv. Aeration and Agitation: Aeration and agitation are important factors that influence the production of biosurfactants as both facilitate the oxygen transfer from the gas phase to the aqueous phase. It may also be linked to the physiological function of microbial emulsifier, it has been suggested that the production of bioemulsifiers can enhance the solubilization of water insoluble substrates and consequently facilitate nutrient transport to microorganisms. Adamczak and Bednarski observed that the best production value of the surfactant (45.5g/l) was obtained when the air flow rate was 1vvm and the dissolved oxygen concentration was maintained at 50% of saturation.
v. Salt concentration: Salt concentration of a particular medium also had a corresponding effect on the biosurfactant production as the cellular activities of microorganisms are affected by salt concentration. Nevertheless, contrary observations were noticed for some biosurfactant products which were not affected by concentrations up to 10% (weight/ volume) although slight reductions in the CMC were detected.

III. MATERIALS AND METHOD

A. Materials

i. Crude oil sample gotten from BOMU oil field in the Niger Delta region of Nigeria
ii. Bacillus Subtilis (Bacteria Used)
iii. Calcium Oxide (Nano Particle used)
iv. CO_2 gas

B. Equipment

i. Air coolant
ii. Redwood Viscometer
iii. Weighing Balance
iv. Pycnometer
v. Stop watch
vi. Beaker
vii. Pensky-Martens Flash Point Tester
viii. Enhanced oil recovery laboratory setup

C. Method

A. Bacterial Preparation

Bacteria (Baccilus Subtilis Species) Preparation

The bacteria used for this experiment is prepared through the following means;

Reagents: The reagents include normal saline for serial dilution process. Ethanol, hydrogen peroxide, Kovac reagent, crystal violet, phenol red, Tetramethyle-phenylendiaminedihychrochloride and biritt reagents. e.t.c

Media used for the cultivation of bacillus species: Nutrient Agar (NA) medium was used to cultivate bacillus on total heterotrophic bacteria (T.H.B) according to manufacturers specification. The medium is a general purpose media for Bacteria. Two Nutrient broths (NB) for proliferation of bacteria (Bacillus Subtilis).

Preparation of Bacillus species: Serial dilution procedure as described by Obire. O and Wemedu S.A (1996), Ofunne (1999), and Csuro S (1999) was employed for the cultivation of known bacteria (Bacillus Subtilis). About 1 gram of soil sample was transferred into 9ml of sterile normal saline, separately to obtain a mixture dilution of 10^1. The mixture was then diluted through a ten-fold serial dilution process to a maximum of 10^-5. About 0.1ml of the selected dilution was inoculated separately onto the nutrient Agar plate (NA) in duplicate. The inoculated plates were incubated at 37°C for 24 hours. After incubation period the ensuring colonies suspected to be Bacillus were subculture onto freshly prepared plates of nutrient Agar to obtain a pure culture. The plates were incubated again at 37°C for 24 hours. The incubated selected colonies of bacteria we're late subjected to microscopic examination and biochemical test that proof the real identity of Bacillus species. The test includes catalase test, citrate test, starch hydrolysis, motility test, MRVP test, indole test and sugar fermentation test. The sugars are, glucose, manitol, lactose, xylose and maltose. The isolate was further identified based on microscopic morphology and reaction pattern to biochemical and sugar fermentation test.

Proliferation of Bacillus Species: A code isolate confirmed to be Bacillus species we're retrieved from the slant and inoculated in 1 litre of nutrient broth. The inoculated broth was incubated at 37°C for 24 hours within which the broth attained a high level of turbidity.

Result: Colonies of isolate suspected to be Bacillus species had circular shape, raised elevation, entire margin, big size, shiny appearance, moist texture and white colour. It is a Gram Positive Rod (GPR) ferment glucose, manitol, maltose, xylose, glycerol and non-fermenter of lactose. It is catalase positive, oxidase positive, cifrate positive, motile positive, methyl red positive, but salt tolerant negative, Urease negative, rogesproskan negative and indole negative.

Result of the Biosurfactant Test

Different biosurfactants were produced after the sample collection, isolation, incubation, purification and extraction of the contaminated soil sample. In these work only one of the biosurfactants will be used to enhance oil recovery. These biosurfactants were cultured and produced in the department of microbiology and was transferred to department of petroleum engineering laboratory of Rivers

State University where it was being used for MEOR experiment.

Table 1.1: Cultural and morphological characterization of bacteria isolates [13]

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
HUB 3	Small, smooth, pinkish colonies and round with entire margin	Serratia sp
HUB 4	Whitish, slightly convex with irregular edges and opaque colonies	Bacillus sp

The type of Biosurfactants used for this experiment is: HUB 4, Bacillus Subtilis

B. Density Determination

The pycnometer method was used to calculate the density of every sample of crude oil. The pycnometer was first empty, cleaned, and thoroughly dried before the weight of the dry, empty pycnometer was recorded. The crude oil was then poured into the pycnometer, and the sample's temperature was measured.

The stopper was positioned above the lid, excess liquid on the pycnometer's body was wiped off, and then the pycnometer's new weight with the fluid was recorded.

$$\rho = \frac{Weight\ of\ pycnometer + sample - weight\ of\ empty\ pycnometer}{Volume\ of\ pycnometer} \quad (1.1)$$

Fig 1.1 (a) Weighing Balance Fig 1.1(b) Pycnometer

C. Determination of Viscosity Using CANNON-FENSKE A B C D E F G H I

A Cannon Fenske Capillary viscometer was used to measure the viscosity of each sample that had been created. The sample was added to the viscometer after it had been thoroughly cleaned and dried with the drying agent. The viscometer was then clamped and set in a bath of constant temperature. One portion of the viscometer had a suction tube attached to it, causing the liquid to rise to the higher engraved line when pressure was applied.

The number of seconds it took for the liquid to flow freely from the higher to lower etched lines was noted.

It's efflux time right now. In order to compute the kinematic viscosity, the efflux time was multiplied by the viscometer constant, and the dynamic viscosity was obtained by multiplying the equation below

$$\mu = ctx\rho \quad (1.2)$$

Where;

C = 0.015 t = Time of flow, = Density

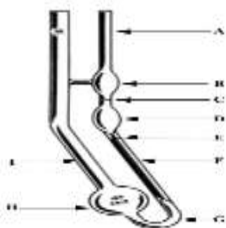


Fig1.2: CANNON-FENSKE A B C D E F G H I Viscosity [11]

D. Cloud and Pour Point Determination

An ice bath was used to calculate the cloud point and pour point. After being correctly labeled, the test jars were filled to the level mark. The mouth of the tube was covered with a closed cork containing the thermometer, and the jar was set into the ice-water bath. In order to check for cloud point, the test jar was swiftly withdrawn from the jacket after a short period of time. The jacket was swapped out.
Until the cloud point appeared, the procedure was repeated, and the temperature at that time was noted as the cloud point temperature.
The sample was left in the bath for as long as it took to notice that the oil surface remained upright for five seconds without sagging. At this point the thermometer was inserted to cool for 10 seconds and the temperature of the oil was taken as the pour point. This procedure was repeated for all remaining samples.

Fig 1.3: Ice Bath [4]

E. Flash point Determination
The Pensky-Martens Closed Cup test, which is used to determine the flash point of flammable liquids, was the method employed in this study to determine flash point. The brass test cup was cleaned and dried before the experiment began. The sample was then poured into the brass cup to the markedly defined level, and the gadget was then properly installed. In order to heat and mix the liquid in the cup, the device was connected to an electric source. At regular intervals, a direct ignition source was placed in front of the ignition point to see whether it would flash. If it did not, the sample was heated, swirled, and tested again until it ignited The flashpoint of the sample was calculated as the lowest temperature at which the crude oil ignited in degrees Celsius.
After discarding the sample, the brass test cup was cleaned and given time to dry. The remaining samples' flashpoint was determined using the same method.

Fig 2.4: Pensky-marten Flash Point Tester [13]

F. API Gravity Determination

The samples' gravities were directly determined using a hydrometer. This procedure involved adding crude oil to the cylinder, using a thermometer to gauge the liquid's temperature, and recording that reading. A continuous floating in the cylinder's center was seen when the hydrometer was cautiously and slowly lowered into the sample from the cylinder's side. The hydrometer was stabilized before taking note of the location where the liquid's surface contacted the stem. The fluid's specific gravity is at this stage.

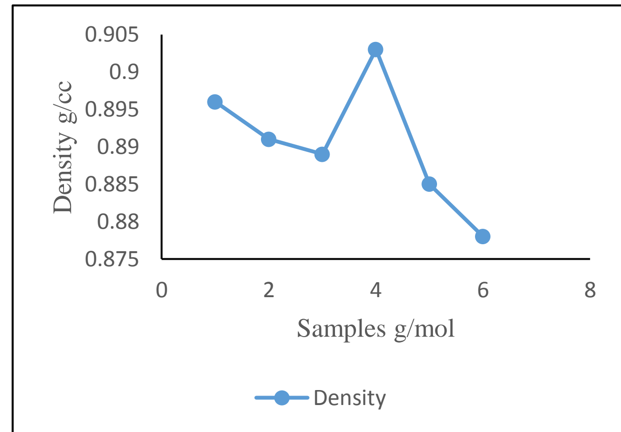
$$API^{o} = \frac{141.5}{S.G} - 131.5 \quad (1.3)$$

G. Microbial Enhanced Oil Recovery Setup

Referring to Fig 1.5, the setup used for this is a laboratory set up for enhanced oil recovery. The setup is made up of a number of items that represent the setup of a well head, including a carbon dioxide cylinder (CO2) that serves as the reservoir pressure, a 12-liter metal tank that serves as the reservoir, a pipe that connects to the tank, and tap handles (stands at the well head valve) along its line. 3 liters of crude oil and 7 liters of water (the carrier fluid) were measured and combined in a bucket before being placed into the tank after the crude oil's characteristics had been examined. The flow line and tank are clear of all liquids and debris, and the pressure vessels were securely shut.
After that, the tank was filled with the crude oil and water mixture and let to settle. The tap handles were likewise opened, and the pressure valve was released at a consistent pressure at the start of the stopwatch. The tap handles and pressure valves were reset to their closed positions at precisely 10 seconds. After 10 seconds, the amount of recovered water and crude oil was measured and recorded. The physical characteristics of a sample of the recovered crude oil were examined. Returning the crude oil and water mixture to the tank, 0.02 liter of bacteria was added, and the mixture was given 24 hours to respond. The same process used to recover the crude and water was repeated after 24 hours. The above-mentioned process was used to assess the amount of crude oil and water recovered after the tank had been filled with water, bacteria, and 5g of calcium oxide. The experiment was repeated for every participant and properly analyzed.

Fig 1.5: Microbial Enhanced Oil Recovery Setup

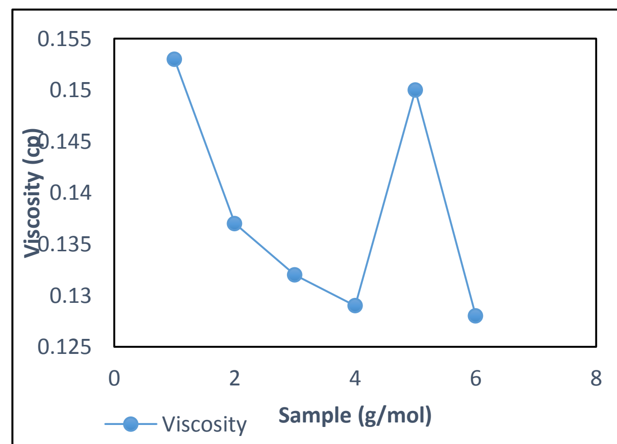
IV. RESULTS AND DISCUSSION



A. Result for Density

Fig 1.6: Graph of Density Vs Samples

From fig 1.6, it is evident that the crude is light with densities ranging from 0.885g/ml to 0.903g/ml. Sample 6 was the heaviest with a density of 0.903g/ml while sample 8 is the lightest at 0.885g/ml. In general, the crude sample itself is light with initial density of 0.897g/ml, the presence of the nanoparticle and biosurfactant slightly increased its densities but still very negligible.



B. Result for Viscosity

Fig 1.7: Graph of Viscosity Vs Samples

From fig 1.7, sample A was recorded to be the most viscous with about 0.153 closely followed by sample E at 0.150cp. Samples B, C, D had viscosities less than half of sample A, this change in Viscosity can be attributed to the introduction of the nanoparticle to the samples. Sample E showed a spike in Viscosity against the trend from samples B to D, these can be attributed to other properties alike. As compared to quality standards, the crude is light and has Viscosity less than 1cp.

C. Result for API Gravity

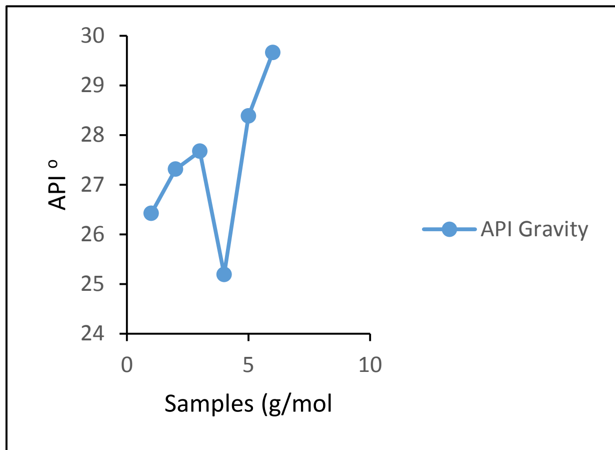


Fig 1.8: Graph of API vs Samples

Figure 4.3 shows the API Gravity of the samples; the API gravities range from 25.19° API (sample D). The API gravity of a product is a measurement of how heavy or light it is in compared to water. If a product's API is less than 10, it is heavy and will sink in water, but if it is greater than 10, it will float. Specific gravity is calculated by dividing the density of a particular object by the density of water. The API gravity of a product is a measurement of how heavy or light it is in compared to water. If a product's API is less than 10, it is heavy and will sink in water; if it is greater than 10, it will float. Specific gravity refers to the density of a specific object divided by the density of water, or the comparison between the density of an equal volume of liquid and water at a specific temperature. The link is that the API gravity is higher when the specific gravity is smaller. It is a comparison of the density of an equal volume of liquid and water at a given temperature. The link is that the API gravity is higher when the specific gravity is smaller.

D. Result for Recovered Volume

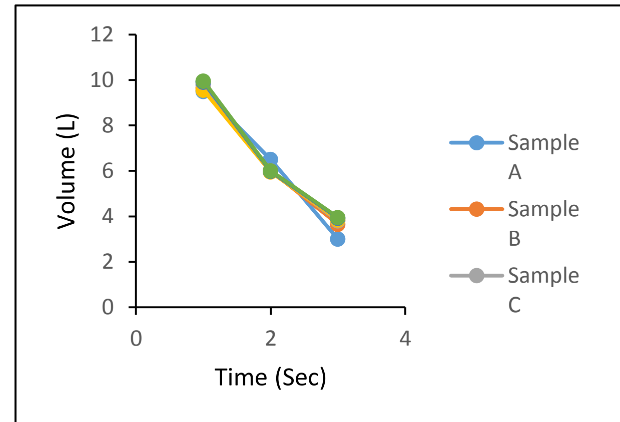


Fig 1.9: Graph of Volume of Oil Recovered Vs Time

Figure 4.4 compares the total recovery volume, water cut oil recovered. The total volume of the recovery for the various samples was obtained at the same temperature and pressure, with the same quantity and qualities of samples. The total recovery volume of each sample varies, as does the amount of oil recovered. According to the graph above, sample D, sample E have the most volume recovered, while sample A, B and sample C have the least volume recovered. Sample E and sample F had the most oil recovered, followed by sample D and sample B, with sample A having the least amount of oil recovered.

V. CONCLUSION AND RECOMMENDATION

A. Conclusion

The Practical work was aimed at the enhancement of BOMU oil field undergoing microbial Enhance Oil recovery using microorganisms (Bacillus subtilis) Calcium

Oxide as a nanoparticle to help fasten the recovery of the crude.

Biosurfactants was observed to emulsify hydrocarbons, reduce the interfacial tension and enhance oil production of the test crude. Factors influencing biosurfactants production are the nature of carbon source, nitrogen source, temperature, aeration and pH. The above result demonstrates that biosurfactants produced by bacillus sp grown on hydrocarbon can be used actively in enhancing oil production. The effects of nanoparticles and Nano-fluid on interfacial tension, physical properties of the crude oil and wettability alteration are the important mechanisms in this study. The use of nanoparticles (calcium oxide) in surfactants solution helps to change the rock wettability, the properties of the crude oil, reduces the interfacial tension and equally enhance oil production.

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

i. The interfacial surface tension of the crude oil was reduced when biosurfactant was added
ii. Sample A which was crude and water, had a high volume of recovery but a lower volume of crude oil recovered
iii. Sample B and sample E are samples containing crude oil and Bacillus biosurfactants, they have a low recovery compared to others sample but they also recovered more crude than sample A
iv. Sample C containing 5g and 20g nanoparticles (calcium oxide) with 20ml of Bacillus sp respectively; they recovered more oil than sample B and sample E which means that the addition of the nanoparticles (calcium oxide) help to recovered more oil
v. Sample D which is the sample containing Bacillus and 10g of nanoparticles (calciun oxide) has the highest total recovery and the highest oil recovery which means that with the help of 10g of calcium oxide makes the biosurfactants more active and more effective in enhancing oil production of the test crude sample.

B. Recommendation

Following the huge capital investment in employing chemical, thermal and gas as some alternate enhanced oil recovery methods, effort should be made towards field trials as mostly every oil well is a good candidate for Microbial Enhanced Oil Recovery (MEOR).

Therefore, from this work and practical, the following recommendations are made:

i. The use of Nanoparticles, Nano-fluid and Nanotechnology is a welcomed development in the energy industry; they will rapidly increase the production/recovery of oil when put into practice in the industry
ii. More microbes should be tested to know the very best type in enhancing the production of crude oil
iii. The use of microbes in the petroleum industry to enhance oil production will be a great benefit because it has been proof to be cost effective
iv. The effect of microbes should be investigated more to know if there will be any effect on the crude properties and the reservoir.

VI. Appendix

Table 2: Presentation of the Experimental Design

Sample Name	Additives
Sample A	Pure crude oil
Sample B	Sample A + 20ml bacillus spp
Sample C	Sample A + 20ml spp + 5g CaO
Sample D	Sample A + 20ml spp + 10g CaO
Sample E	Sample A + 20ml spp + 15g CaO

Table 2: Crude oil properties for sample A

Properties @ 29°C	Values
Density	0.905g/ml
API gravity	27.31°
Specific gravity	0.891
Viscosity	0.198cp

Table 3: Crude oil properties for sample B

Properties	Values
Density	0.903g/ml
API gravity	27.66°
Specific gravity	0.889
Viscosity	0.190cp

Table 4: Crude Oil Properties for Sample C

Properties	Values
Density	0.900g/ml
API gravity	27.85°
Specific gravity	0.888
Viscosity	0.179cp

Table 5: Crude Oil Properties for Sample D

Properties	Values
Density	0.896g/ml
API gravity	28.21°
Specific gravity	0.886
Viscosity	0.170cp

Table 6: Crude Oil Properties for Sample E

Properties	Values
Density	0.895g/ml
API gravity	28.57°
Specific gravity	0.884
Viscosity	0.163cp

Table 7 Summary for Result for Enhanced Recovery Processes

Sample	Density (g/ml)	Viscosity (cp)	API Gravity (°API)
A	0.896	0.153	26.42
B	0.891	0.137	27.31
C	0.889	0.132	27.67
D	0.903	0.129	25.19
E	0.885	0.150	28.38

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