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## Cover Page Footnote

We acknowledge the MEOR team in OGRINDO ITB and Reservoir Fluid Analysis Laboratory, Oil Engineering, Bandung Institute of Technology for the help during the research.

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## Abstract

Oil production in Indonesia has been declining since 2002, and this decline is incompatible with the high oil consumption in Indonesia. As such oil production in Indonesia should be improved. Biostimulation in microbial enhanced oil recovery involves environmental modification to stimulate microbial growth within a reservoir by adding limiting nutrients. Variation in C, N, and P concentrations injected to reservoirs can induce different responses from indigenous microbes and increase oil recovery. In this study, molasses, urea and diammonium phosphate were used as nutrients injected to a reservoir for biostimulation. Biological, physical and chemical characteristics after biostimulation were observed and bacterial growth was monitored up to 42 days. The physical characteristics observed were pH, oil viscosity, and interfacial tension. Gas chromatography mass spectrometry was performed to determine the chemical characteristics of oil. Results showed that the addition of nutrients at various concentrations yielded different production patterns of metabolites. The addition of urea and diammonium phosphate induced biosurfactants overproduction and increase hydrocarbon degradation of by bacteria. Therefore, hydrocarbons were degraded for the first 14 days, and polymerized again on days 14 to 42.

*Keywords: biostimulation, nutrition, molasses, diammonium phosphate, urea*

## Introduction

The decline in Indonesia's oil production and an increased domestic demand for oil have promted Indonesia to become a net oil importer since 2002. As such, a solution is needed to reduce the amount of oil imported by increasing Indonesia's oil production through enhanced oil recovery (EOR) technology [1]. For instance, microbial enhanced oil recovery (MEOR) is an environment-friendly and low-operating cost treatment technology to increase oil production by using microbes. An examples of MEOR techniques is biostimulation that involves the injection of limiting nutrients to a reservoir [2].

The main principle of biostimulation is adding nutrients, such as carbon, nitrogen, and phosphorus to promote microbial propagation and metabolites production within a reservoir. These added nutrients increase the growth of indigenous microbes and change the physico-chemical characteristics of oil in reservoir leading to an increase in oil recovery. C, N, and P are essential elements for microbial growth, but their availability in the environment is limited, so they must be added to increase microbial growth [3]. The carbon source commonly used in MEOR is molasses. Molasses is a relatively cheap carbon source that can more effectively stimulate microbial growth than other carbon sources [4]. The addition of molasses can also trigger the bacterial production of metabolites such as organic acids, solvents, gases, and biosurfactants [5]. Nitrogen sources commonly used in MEOR are are fertilizer (contain nitrogen, phosphorus, and pottasium), urea, and diammonium phosphate ($(NH_4)_2HPO_4$). These nitrogen sources are soluble in water so they would be easily used by bacteria [6,7].

Variations in nutrient concentrations can generate different stimulation responses from indigenous microbes. Microbes supplied with appropriate nutrients can produce biosurfactants, solvents, gases, organic acids, and biopolymers. These microbes can also break up long chains of paraffin and asphaltenes, modify the wettability of the reservoar rock, decrease the viscosity and density of oil, and increase pressure within the pore network of the rocks. The success of MEOR has been reported; in particular, the growth of indigenous bacteria is stimulated by supplying nutrients, and most of the treated reservoirs have shown positive results [3]. However, the effect of added nutrient concentrations on

indigenous microbial stimulation should also be determined. In this research, different nutrient concentrations were used to examine their effects on the growth of indigenous microbes and the physical and chemical properties of oil samples.

## Materials and Methods

**Determination of cell growth.** A total plate count (TPC) method was used to calculate the total oil derived bacterial growth in each treatment after incubation. Nutrient agar media were used to determine the growth of facultative anaerobes and anaerobes bacteria [6]. A pour plate method used to isolate bacteria; in this procedure, the media were serially diluted from $10^0$ to $10^2$ and gradually increased based on the basis of the observations of bacterial growth [5].

**Screening of nitrogen sources.** In this procedure, 200 mL of media with 93% brine water (v/v), 7% oil (v/v), 5% molasses (w/v), 0.6% $NaNO_3$ (w/v) and 0.5% nitrogen sources (urea + $KH_2PO_4$, DAP, NPK w/v) were placed in sterile bottles. Anaerobic conditions were made by adding nitrogen gas to the bottle and closing it tightly. The samples were then incubated at 50 °C for 9 days without agitation and sampling was carried out every 3 days [5]. Table 1 represents the experimental design for screening of nitrogen sources by using 5% concentration of molasses.

**Variations in nutrients for biostimulation.** In this procedure, 200 ml of media containing 93% brine water, 7% crude oil, and nutrients was sterilized (Table 2). The mixture of the media was transferred to a 250 ml sterile bottle and then added with nitrogen gas to provide an anaerobic condition. Optimization was carried out for 6 weeks in an incubator at 50 °C without agitation for 6 weeks [5]. The composition of nutrients added was summarized on Table 2. Therefore, $NaNO_3$ + brine as the control + $NaNO_3$ sample was examined to determine the effect of $NaNO_3$ addition on the brine sample. Sampling was carried out every 7 days for yhe TPC, pH measurement, and analysis of oil characteristics.

**Measurement of oil characteristics.** Interfacial tension (IFT) and oil viscosity were measured three times, namely on days 0, 14, and 42 of incubation day. The nutrients loaded to the media on day 0. IFT was measured using a Du Nuoy tensinometer at 50 °C. Oil viscosity was determined with an Oswald viscometer at 50 °C [8]. Oil fraction was determined through gas chromatography-mass spectrometry (GC-MS). Before being analyzed, the oil samples were given n-hexane solvents. Data were then processed using the MSDCHEM 5975 software.

**Table 1. Nitrogen Sources for Screening Design**

| Carbon source | Nitrogen source |
|---|---|
| Molasses 5% | NaNO3 (0.6%) +$(NH_4)$2HPO4 0.5% |
| Molasses 5% | NaNO3 (0.6%) + fertilizer 0.5% |
| Molasses 5% | NaNO3 (0.6%) + Urea 0.5%+K2HPO4 0.5% |

**Table 2. Nutrition Variations for Biostimulation**

| Treatment | Molasse (%) | Urea (%) | DAP (%) |
|---|---|---|---|
| 1 | 0 | 0.5 | 0.5 |
| 2 | 2 | 0.2 | 0.2 |
| 3 | 2 | 0.8 | 0.2 |
| 4 | 2 | 0.2 | 0.8 |
| 5 | 8 | 0.8 | 0.8 |
| 6 | 8 | 0.2 | 0.2 |
| 7 | 8 | 0.8 | 0.2 |
| 8 | 8 | 0.2 | 0.8 |
| 9 | 8 | 0.8 | 0.8 |

## Results and Discussion

**Screening of nitrogen and phosphorus sources.** Figure 1 shows the results of nitrogen and phosphorus screening for the growth of consortium anaerobes (1a) and facultative anaerobes (1b) derived from oil. The added nutrients were a combination between nitrogen and phosphorus (Table 1). The growth of anaerobic bacteria (1a) under the nutrient combinations exhibited the same patterns. Anaerobic bacteria reached their maximum growth on day 6 of incubation time and decreased on day 9. Because the nutrient source was limited on day 9 and could not induce the bacteria to degrade more complex nutrient sources; as such, bacterial growth decreases [9].

In facultative anaerobic bacteria (1b), a different growth pattern was observed between the nutrients given. The facultative anaerobes in DAP and urea have had the same growth patterns as those of anaerobic bacteria. Conversely, the addition of NPK undesirably reduced bacterial growth on the first 6 days of incubation time. Therefore, DAP and urea were chosen as nitrogen sources because both could more effectively maintain bacterial growth than fertilizer. Organic nitrogen sources, such as urea, can be easily used by bacteria for their growth on complex substrates, such as hydrocarbons, which cannot be degraded easily [8]. In addition, urea can be directly converted by bacteria into ammonia via urease so that it can be used easily for amino acid synthesis [10]. While DAP is an inorganic nitrogen usually utilized for MEOR applications because it can be easily decomposed in water can increase indigenous bacterial growth and can significantly decrease IFT and viscosity [5].

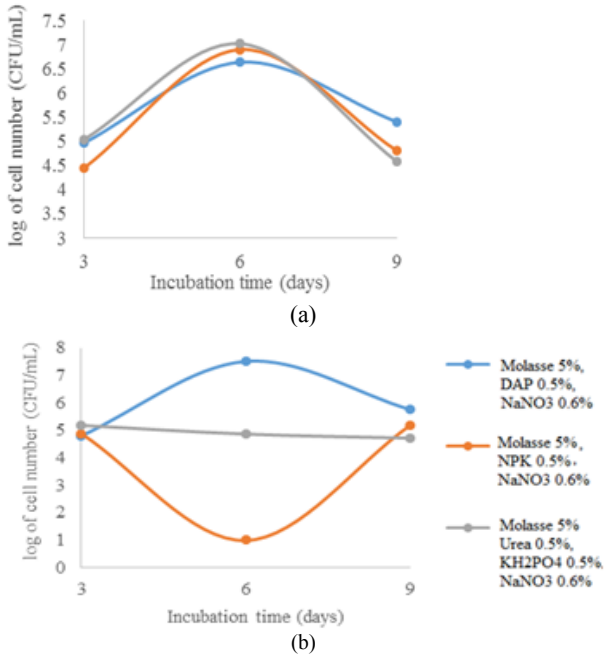


**Figure 1. Growth Pattern of Anaerobic Bacteria (a) and Facultative Anaerobic Bacteria (b) in N Source Screening Treatment**

**Effects of nutrients on anaerobic and facultative anaerobic bacterial growth.** Figure 2 shows the anaerobic microbial growth pattern in the presence of the given nutrients (Table 2). The cell growth in the control and control + $NaNO_3$ samples was slightly enhanced compared with that in the samples with the added nutrients. Therefore, $NaNO_3$ addition could increase bacterial growth compared with that of the control even when the increase in bacterial growth was 10-fold to 100-fold [11]. Upon the addition of nutrients, the number of cells could increase up to 1000-fold. Figures 2 (b), (c), (d), and (e) illustrate that molasses can increase the growth of indigenous bacteria because molasses contained 48%–56% simple sugars and few microelements, which can be easily used by bacteria [13].

At the beginning of incubation, the number of bacteria increased because growth is induced by the addition of molasses [4]. In the control and control + $NaNO_3$ treatment, the number of cells also increased in the initial incubation time presumably because bacteria in the media have adapted to oil as nutrients [6]. After incubation was extended for 14 days, the number of bacterial cells decreased. After the molasses were consumed, indigenous bacteria used hydrocarbons as a carbon source. The hydrocarbon fraction used first by the bacteria was the light fraction because it can be easily degraded [8]. The number of bacteria that could not degrade this fraction decreased. Afterward, the number of bacterial cells increased because bacteria used simple hydrocarbons (light fraction) until the 28th day of incubation.

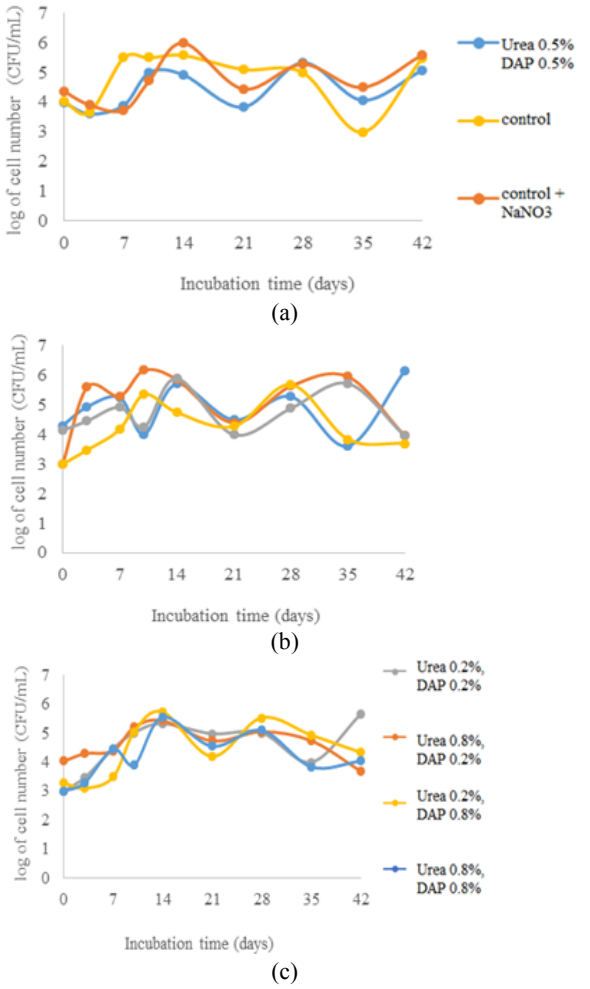


**Figure 2. Growth Pattern of Anaerobic Bacteria After Added NaNO3 (0.6%) and Molasses: (a) 0% (b) 2%, (c) 8% After 42 Days of Incubation**

When the light fraction of the oil was fully consumed, the number of cells decreased after 28 days of incubation because bacteria preferred the heavy fraction of hydrocarbons to the light fraction. The number of bacteria that could degrade simple hydrocarbons decreased, and they were replaced by bacteria that could degrade complex hydrocarbon substrates because of the change in the substrate. The number of cells since day 28 increased because bacteria that can degrade the heavy fraction of hydrocarbons have adapted to their new nutrient source and can use the fraction to grow [6].

Overall, bacterial growth patterns in all the treatments were similar because all the indigenous bacteria switched their metabolic pathway based on nutrient availability in media. Nutritional change patterns between the injected nutrients and hydrocarbons in oil occurred in all the treatments. However, the treatment favored a different metabolite production pattern. Some of the injected nutrients could induce the overproduction of metabolites that could improve oil recovery.

Bacterial growth patterns could not be considered a single parameter influencing the success of biostimulation because several factors, such as the composition of brine and molasses in each treatment, could not be controlled. Brine and molasses were the complex media used in this research. Both nutrients added to each treatment contain different compositions, although this composition difference is not remarkably similar with that of nutrients added to the media [14].

**Effect of nutrients on the pH of media.** Figure 3 shows the decrease in the initial pH of the media added with molasses compared with that of the treatment without molasses. The pH decreased for 14 days as the cells substantially grew at the beginning of the incubation period. This phenomenon occurred because the amount of acid produced by bacteria in the early incubation period was incredibly high. Most bacteria found in oil are fermentative bacteria that can produce gas and acid [4]. After 14 days, pH increased in almost all the treatments and remained constant until the end of incubation. This increase in pH could be caused by the accumulation of alkaline compounds produced via bacterial cell lysis [6]. The decrease in pH in the control treatment (Figure 3a) was not as much as that in the treatments added with nutrients because of the low growth of bacteria in the control treatment.

**Effect of nutrients on IFT between oil and brine water.** The degree of reduction in IFT in the treatment with added nutrients was higher than that in the control sample (Figure 4). In the treatment with the addition of $NaNO_3$ only, IFT also decreased, indicating that $NaNO_3$ is an important nutrient in inducing biosurfactant production by bacteria. Biosurfactants are active compounds produced on a microbial cell surface or excreted, thereby reducing surface tension and IFT [11]. For the treatment with the added nutrients, IFT decreased significantly and almost reached the degree observed in the control sample. This result showed that the addition of nutrients in the form of molasses, urea, and DAP could induce bacterial growth, thereby increasing biosurfactant production by bacteria. The highest IFT reduction was 28.6 dyne/cm to 13.1 dyne/cm in the

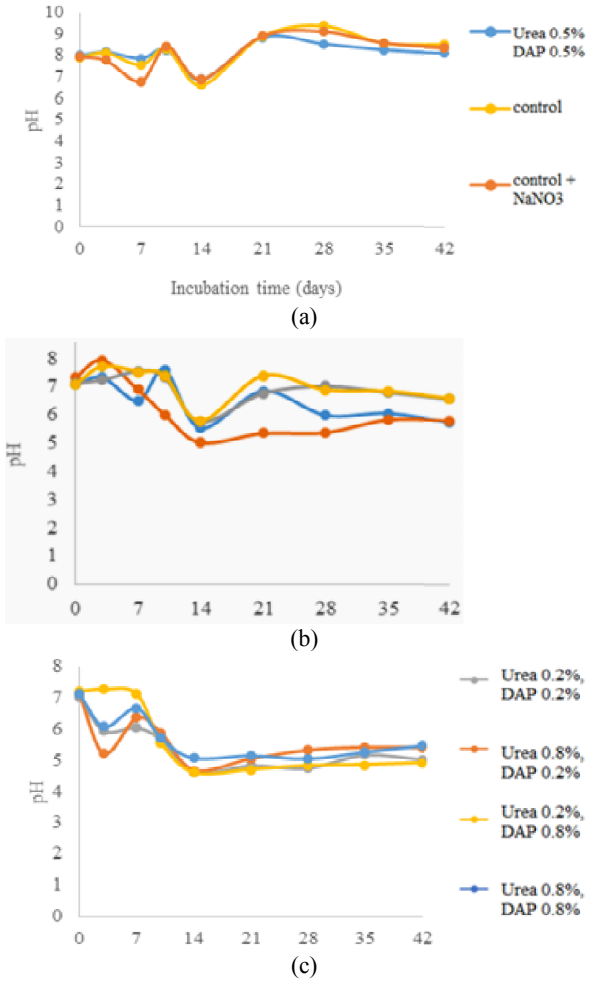


**Figure 3. Growth Pattern of pH After Added NaNO3 (0.6%) and Molasses: (a) 0%, (b) 2%, (c) 8% After 42 Days of Incubation**

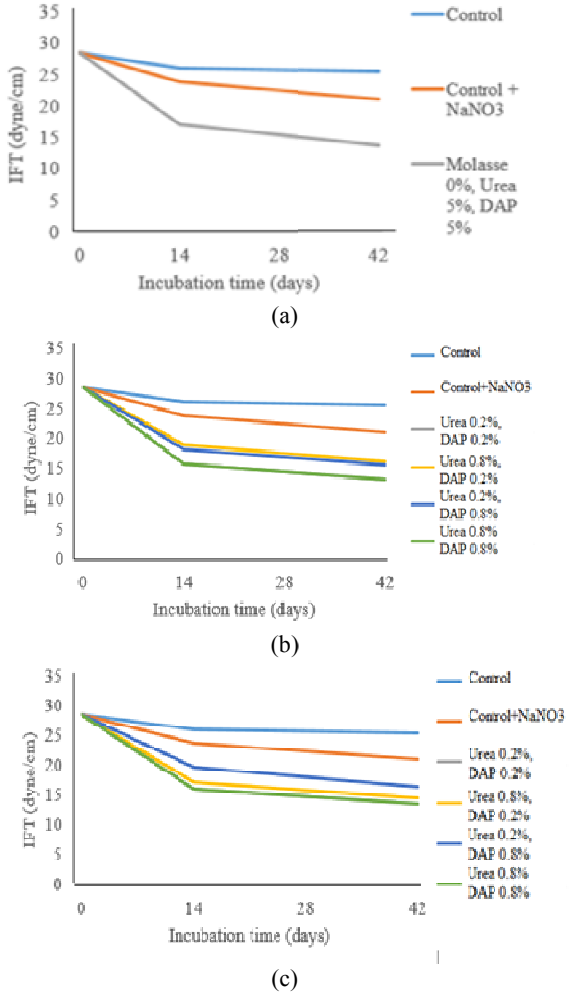


**Figure 4. Growth Pattern of IFT After Added NaNO3 (0.6%) and Molasses: (a) 0%, (b) 2%, (c) 8% After 42 Days of Incubation**

addition of 2% molasses, 0.8% urea, and 0.8% DAP. This result suggested some suitable nutrients for biosurfactant production by indigenous microbes.

**Effect of nutrients on oil viscosity.** Figure 5 illustrates a decrease in oil viscosity during incubation compared with that in the control sample. This finding confirmed that molasses, urea, and DAP could induce bacterial growth, thereby increasing the degradation of the heavy fraction of oil by bacteria. In accordance with IFT reduction in media + $NaNO_3$, the viscosity of oil with the addition of $NaNO_3$ only decreased. Biosurfactants facilitate bacteria to rapidly use hydrocarbons. In oil biodegradation, the primary role of biosurfactants is to increase the collision frequency between bacteria and oil [11]. Despite the high amount of biosurfactants produced upon the addition of $NaNO_3$, the oil viscosity decreased slightly because the number of bacteria that could degrade hydrocarbons in this treatment was insufficient (Figure 2).

In all the treatments after 42 days of incubation, the decrease in viscosity ranged from 24.33% to 92.02%. The best reduction in oil viscosity was obtained when 2% molasses (*v*/*v*), 0.8% (*w*/*v*) urea, and 0.8% (*w*/*v*) $(NH_4)_2HPO_4$ were added because a high nitrogen concentration considerably influenced cell growth (Figure 2). A high number of cells increased the degradation of hydrocarbons. Bacteria in oil wells can use hydrocarbons as a carbon source and convert them into simple compounds. Hydrocarbons can be degraded by microorganisms under aerobic and anaerobic conditions with various enzymes possessed by hydrocarbon clastic microbes [13]. With degradation, a long oil fraction can be converted into a simple fraction, so oil viscosity decreases.

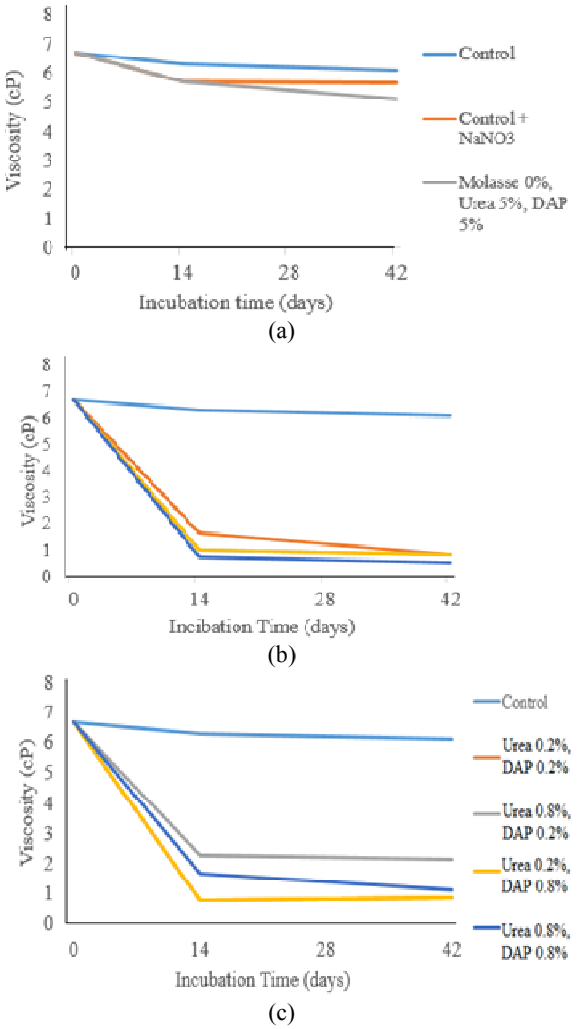


**Figure 5. Oil Viscosity Pattern with Molasses: (a) 0%, (b) 2%, (c) 8% After 42 Days of Incubation**

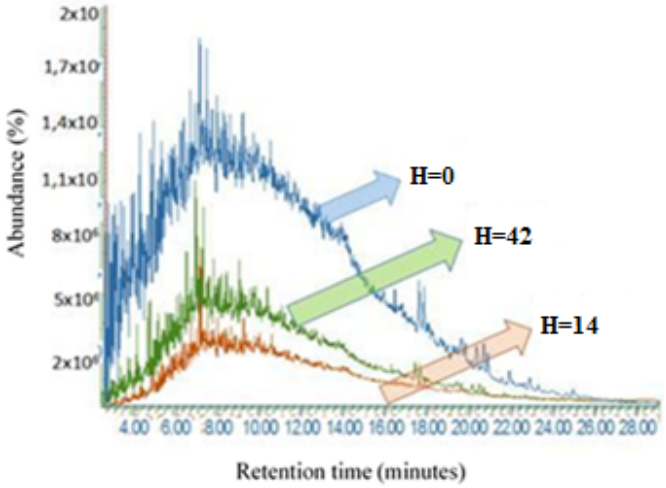


**Figure 6. GC-MS Chromatogram Peak during Incubation Time**

**Change in hydrocarbon fraction.** The viscosity of the oil sample with 2% molasses (*v*/*v*), 0.8% (*w*/*v*) urea, and 0.8% (*w*/*v*) $(NH_4)_2HPO_4$ highly decreased. This sample was analyzed through GC–MS to observe the changes in the hydrocarbon fraction on days 0 (the day the nutrients were loaded), 14, and 42. Figure 6 shows the chromatogram of the hydrocarbon fraction from the treatment with the added nutrients. The peak of the control sample (day 0) was higher than that observed on other days. A high peak indicated that the sample had many heavy hydrocarbon fractions. After 14 days, the peak declined significantly because indigenous microbes grew rapidly so degradation of hydrocarbon were high. The bacteria also changed their preference from a heavy hydrocarbon fraction to a light hydrocarbon fraction that affected oil viscosity. This finding indicated that the hydrocarbons were degraded by bacteria, thereby decreasing the abundance of heavy fractions and converting them to a simple fraction. This change could be observed in the loss of heavy fractions, such as cyclohexane-2,4-diethyl-1-methyl, 2-butylidencyclopentane, 2,6-dimethylbicyclo-[321]-octane, and 3-dimethyl-2-propilidemidazolidine, on days 14 and 42 (Table 3).

However, on day 42, the peak slightly increased, showing that the hydrocarbon fraction polymerized to become a more complex fraction than day 14. Hydrocarbon polymerization can occur mainly in unsaturated hydrocarbons to form polymers with large molecular weights [12]. This finding could be supported by a slight decrease in oil viscosity from days 14 to 42 in this treatment.

**Table 3. Hydrocarbon Detected During Incubation**

| No | Compounds | Detected Hydrocarbon | | |
|---|---|---|---|---|
| | | Day-0 | Day-14 | Day-42 |
| 1 | Cyclohexane, 2,4-diethyl-1-methyl- | + | - | - |
| 2 | Cyclohexane, 1,1,3,5-tetramethyl-Cyclohexane, | + | - | - |
| 3 | 2-Butylidenecyclopentane | + | - | - |
| 4 | 1,3-dimethyl-2-propylidenimidazolidine | + | - | - |
| 5 | Cyclohexane, 1,1,2,3-tetramethyl- | + | - | - |
| 6 | 1-Hexadecyne | + | - | - |
| 7 | 2,6-Dimethylbicyclo[3.2.1]octane | + | - | - |
| 8 | 1-Decene, 10-bromo- | + | - | - |
| 9 | 2-methyldecalin (probably trans) | + | - | - |
| 10 | (Z)-1-Ethyl-2-(1,2,2-trimethylpropylidene)cyclopropane | + | - | - |
| 11 | Cis-1-ethinyl-2-methyl-1-cyclohexanol | + | - | - |
| 12 | 1-Methyladamantane | + | - | - |
| 13 | Naphthalene, decahydro-2,3-dimethyl | + | - | - |
| 14 | Naphthalene, decahydro-1,6-dimethyl- (CAS) | + | + | + |
| 15 | Decahydro-4,4,8,9,10-pentamethylnaphthalene | + | + | + |
| 16 | amorphane-B | + | + | + |
| 17 | (4aRS)-3,4,4a,5,6,7,8,8a-Octahydro-5,5,8a-trimethyl-21H | + | + | + |
| 18 | cis,trans-1,6-Dimethylspiro[4.5]decane | - | + | + |
| 19 | Benzene, cyclohexyl- (CAS) | - | + | + |
| 20 | 1H-Indene, 2,3-dihydro-1,1,4,6-tetramethyl- (CAS) | - | + | + |
| 21 | Naphthalene, 1-ethyl- | - | + | + |
| 22 | Naphthalene, 1,2,3,4-tetrahydro-5,6,7,8-tetramethyl- | - | + | + |
| 23 | 2,2,6.Beta.,7.Alpha.-Tetramethyl-icyclo(4.3.0)Nonan-7.Beta.-Ol | - | + | - |
| 24 | salvialane | - | + | - |
| 25 | Naphthalene, 1,4,6-trimethyl- | - | + | - |
| 26 | trans-Decalin, 2-methyl- | + | - | - |

Notes= (+):detected , (-): not detected

## Conclusion

The addition of $NaNO_3$ can induce bacterial growth, thereby reducing oil viscosity and IFT. The addition of molasses at any concentration also stimulates indigenous bacterial growth. Similarly, the addition of urea and DAP causes biosurfactant overproduction and increases hydrocarbon degradation by bacteria. The nutrients and their concentrations that yield the best IFT and oil viscosity reduction are 2% molasses (*v*/*v*), 0.8% urea (*w*/*v*), and 0.8% DAP (*w*/*v*). The GC–MS peak shows that hydrocarbons are degraded during the first 14 days of incubation and polymerized on days 14 to 42 in oil amended with nutrients.

## Acknowledgments

We acknowledge the MEOR team in OGRINDO ITB and Reservoir Fluid Analysis Laboratory, Oil Engineering, Bandung Institute of Technology for the help during the research.

## References

[1] Dudley, B. 2019. BP Stastitical review of World Energy. 8–12.

[2] Youssef, N., Elshahed, M.S., Mclnerney, M.J. 2009. Chapter 6: Microbial processes in oil field: culprits, problems, and opportunities. Adv. Appl. Microbiol. 141–251, http://dx.doi.org/10.1016/s0065-2164(08) 00806-x.

[3] Lazar, I., Petrisor I.G., Yen, T.F. 2007. Microbial enhanced oil recovery (MEOR). Oil Sci. Technol. 25: 1353–1366, http://dx.doi.org/10.1080/10916460701287714.

[4] Canul-Chan,M., Chable-Naal, J., Rojas-Herrera, R. 2017. Hydrocarbon degradation capacity and population dynamics of a microbial consortium obtained using a sequencing batch reactor in the presence of molasses. Biotechnol. Bioproc. E. 22: 170–177, http://dx.doi.org/10.1007/s12257-016-0499-4.

[5] Gao, P., Li, G., Dai, X., Wang, H., Zhao, L., Chen, Y., Ma, T. 2013. Nutrients and oxygen alter reservoir viochemical characters and enhance oil recovery during biostimulation. World J. Microbiol. Biotechnol. 29: 2045–2054, http://dx.doi.org/10.1007/s11274-013-1367-4.

[6] International Plant Nutrition Institute (IPNI). 2016. Diammonium Phospate. www.ipni.net/diammonium_phospate.

[7] Chorom, M., Sharifi, H.M. 2010. Bioremediation of crude oil–polluted soil by application of fertilizers. Iran. J. Environ. Health Sci. Eng. 7(1): 319–326, http://dx.doi.org/10.4172/2157-7463.1000198

[8] Suthar, H., Hingurao, K., Desai, A., Nerurkar, A. 2019. Selective plugging strategy based microbial enhanced oil recovery using bacillus licheniformis TT33. J. Microbiol. Biotechnol. 19: 1230–1237.

[9] Margesin, R., Schinner, F. 2001. Bioremediation (natural attenuation and biostimulation) of diesel oil-contaminated soil in an alpine glacier skiing area. Appl. Environ. Microbiol. 67: 3127–3133.

[10] Moat, A.G., Foster, J.W., Spector, M.P. 2012. Microbial Physiology. 4th Ed. Huntington: John Wiley & Sons, pp. 407–410.

[11] Aboim, J.B., Oliveira, M. 2001. Optimization of light intensity and NaNO3 concentration in amazon cyanobacteria cultivation to produce biodiesel. Mol. 24: 2326, http://dx.doi.org/10.3390/molecules 2412 2326.

[12] Kamigato, M., Satoh K. 2015. Bio-based hydrocarbon polymers. Encyclopedia of polymeric nanomaterials. Springer, Berlin, Heidelberg.

[13] Sierra-Gracia., de Oliviera, M. 2009. Microbial degradation hydrocarbon: Efforts to understand biodegradation in oil reservoirs. Biodegrad. Eng. Technol. 53, http://dx.doi.org/10.5772/55920.

[14] Peyman, R., Fattahi, M., Mohammad, M., Morteza, S., Ayatollahic, D.S. 2019. Effect of water salinity on oil/brine interfacial behaviour during low salinity waterflooding: A mechanistic study. Pet. 5: 367–374, http://dx.doi.org/10.1016/j.petlm.2019.03.005.