

Oil recovery test using bio surfactants of indigenous bacteria in variation concentration of carbon source

B Yudono¹, W Purwaningrum², S P Estuningsih³, S Kaffah⁴

^{1,2,4}Department of Chemistry, University of Sriwijaya, Palembang, Indonesia

³Department of Biology, University of Sriwijaya, Palembang, Indonesia

Email: yudonob@hotmail.com

Abstract. Recovery tests of crude oil by using bio surfactant of indigenous bacteria *Pseudomonas peli*, *Pseudomonas citronellolis*, *Burkholderia glumae* and *Bacillus firmus*. The bio surfactants were prepared with the variation concentrations of molasses carbon source; 0, 5, 10, 15, 20, and 25 %. The results showed that 10 g samples, which concentration 18.64% TPH could be dissolved in the bio surfactant 10%. Optimally in the molasses carbon source concentrations for each bacterium at 5, 10, 20 and 15 % with oil recovery as much as 31.92, 17.65, 22.32, and 14.38 % respectively. Oil components which extracted by bio surfactant were analyzed by using GLC (Gas Liquid Chromatography). The bio surfactants of *Pseudomonas peli* could dissolve oil fraction temperatures; 139.85; 144.69; 149.98; 155.03; 174.22 °C, *Pseudomonas citronellolis* could dissolve oil fraction temperatures; 139.13; 142.64; 147.99; 155.03; 159.85; 164.50 °C, *Burkholderia glumae* could dissolve oil fraction temperatures 144.69; 149.98; 155.03; 159.85; 164.50 °C, and *Bacillus firmus* could dissolve oil fraction temperatures; 149.98; 155.03; 158.46; 164.50 °C.

1. Introduction

Petroleum is a natural resource that can be utilized to increase the sources of foreign exchange for the country. The needs of the world including Indonesia in petroleum from year to year increase. The role of oil until now has not replaceable whereas petroleum searches more difficult, as well as its exploitation increasingly expensive. Microbial Enhanced Oil Recovery Technology (MEOR) is an alternative method that is used to retrieve oil reserves are not drawn conventionally performed by utilizing microbes work processes contained in the reservoir which is dominated by bacteria [1].

This method utilizes the activity and bio product (gas, bio surfactant, biopolymers, acids, solvents, biomass) produced by microorganisms [2]. MEOR method utilize microbes to produce bio surfactant, where microbes grown on certain media and certain conditions. Bio surfactants can reduce the surface and interfacial tensions [3]. Bio surfactants consist of molecules of hydrophilic and hydrophobic. The existence of both these groups cause the bio surfactant can reduce the surface tension and interfacial



tension and can form a stable emulsion that can make hydrocarbons soluble in water or otherwise water-soluble hydrocarbons [4]. MEOR method requires only slight modifications to the facilities on the ground, MEOR very suitable for oil reservoir with carbonate rocks, have low toxicity, biodegradability and biocompatibility specificity, selectivity, extreme temperature, pH and salinity [5].

Microbes characteristics which must be owned by a bacterium that is capable of processing MEOR; hydrocarbons (hydrocarbonoclastic), produce bio surfactant, produces gas, small size, barophilic, thermophiles, halophile, not pathogenic, and indigent [6]. Hydrocarbonoclastic bacteria are able to use carbohydrates such as glucose, fructose, and mannitol as a carbon source to support bio surfactant [7]. The concentrations of carbon source are focus in this study. This is because the concentration of carbon source is one that affects bacterial growth and employment in the bio surfactant. Based on research [8] studied the effect of carbon source (stearic acid, oil, and molasses) on the growth of *Pseudomonas peli*. The results showed that the best carbon source is molasses with a percentage of 16.01% at a concentration of 4%.

In this study, the recovery of petroleum tested using bio surfactant of bacteria indigent *Pseudomonas peli*, *citronellolis Pseudomonas*, *Burkholderia glumae* and *Bacillus firmus* by varying the concentration of carbon source molasses 0, 5, 10, 15, 20 and 25%.

2. Research Methodology

2.1. Tools and Materials Research

The tools used in This study is an autoclave, Erlenmeyer 1000 mL, 500 mL Erlenmeyer, Erlenmeyer 250 mL, test tubes, beakers 250 mL, measuring cups 100 mL, a set of GLC-Shimadzu, a set of tools soxhlet, analytical balance, microscopes, incubators, needle ose, petri dishes, filter paper, funnel, beaker glass, pipette, aerator, aerator stone, plastic hose, cotton, aluminum foil, Bunsen, paper labels, paper, mesh, camera, spatula, bowl, mortar, rotary shaker, hot plate, and a magnetic stirrer.

Materials used are bacteria isolates; *Pseudomonas peli*, *citronellolis Pseudomonas*, *Burkholderia glumae*, *Bacillus firmus* , distilled water, K₂HPO₄, peptone, FeSO₄, yeast extract, alcohol 70%, n- hexane technical, NA medium, molasses (taken from the sugar mill waste PT. Cinta Manis, Tanjung Raja, Ogan Ilir, South Sumatra), medium clay and sludge oil obtained from the Village Babat Toman, Banyuasin, South Sumatra [9].

2.2. Stages of Research

2.2.1. Sample preparation

Clay as much as 1 Kg dried in an oven with a temperature 110°C for 3 days. Clay is dried and then ground using a mortar until smooth and sifted so that the clay is still shaped blobs can be separated with clay that has been refined. Clay 700 g and 300 g as much petroleum sludge is then mixed and stirred until homogeneous.

2.2.2. Indigenous bacteria preparation

Each bacterium, namely; *Pseudomonas peli*, *citronellolis Pseudomonas*, *Burkholderia glumae*, *Bacillus firmus* inoculated with a zigzag motion in NA slant agar (Nutrient Agar) aseptically, then incubated at a temperature of 37°C for 24 hours and will be acquired stock cultures and working cultures were ready for use [10].

2.2.3. Preparation of medium zobell

Medium Zobell made with dissolve 5 g peptone, 1 g yeast extract, 0.012 g K₂HPO₄, and 0.01 g FeSO₄ in distilled water by volume solution of 1000 mL. The mixture is boiled over a hot plate and homogenized with a magnetic stirrer. Then the mixture sterilized by autoclaving at 121°C for 15 minutes [10].

2.2.4. Starter bacteria indigen preparation

Bacterial culture is taken as much as 5 ose, then subculture to put into each Erlenmeyer containing 100 ml of medium Zobell, then aerated for 24 hours, after 24 hours as much as 100 mL was added to the mixture until the total volume of 200 mL and the mixture is aerated again until the shortest generation time of bacteria. The shortest generation time for the bacteria *Pseudomonas peli* is 12 hours, the bacteria *Pseudomonas citronellolis* is 9 hours, the bacteria *Burkholderia glumae* which is 12 hours and the bacteria *Bacillus firmus* i.e. 15 hours [8].

2.2.5. Initial measurement TPH

A total of 10 g soil is inserted using the appropriate filter paper. The initial TPH concentration is measured by using soxhletation method, dried in an oven, cooled in a desiccator and weighed.

TPH percentage of petroleum is calculated using the formula:

$$\%TPH = \frac{w_1 - w_2}{w_3} \times 100\% \quad (1)$$

Where:

W₁ = Weight boiling flask and extract petroleum oil (g), W₂ = weight of the empty boiling flask (g), W₃ = weight of the sample (g) [11].

2.2.6. Production of crude bio surfactants

Varying concentrations of molasses 0, 5, 10, 15, 20, and 25%, mixed Zobell media, and then inoculated with indigenous *Pseudomonas peli*, *citronellolis Pseudomonas*, *Burkholderia glumae*, *Bacillus firmus* bacteria respectively. The cultures were incubated by the shortest generation time of each bacterial [8].

2.2.7. Test oil recovery

Erlenmeyer already containing 10 g sample (a mixture of soil and sludge) was added to 200 mL of crude bio surfactant of *Pseudomonas peli* at varying concentrations of molasses 0, 5, 10, 15, 20, and 25%, then aerated with an aerator for 10 days. The mixture is then filtered using a filter paper; the precipitate was washed using distilled water and dried in an oven at 100°C for 24 hours. The precipitate was dried and then weighted. The same treatment was also carried out using *Pseudomonas citronellolis*, *Burkholderia glumae*, and *Bacillus firmus* bacteria. The oil recovery calculated from the difference TPH contents between initial and final samples.

2.2.8. GC analysis

The samples were diluted with n-hexane then injected 2.5 µL into the GC-Shimadzu instruments. Injector temperature is 290°C, temperature hold 40°C for 5 minutes, and then the temperature is increased every minute to the temperature rise to 280°C with temperature step 5°C per minute and hold for 5 minutes. The mobile phase used is Helium [12].

3. Results and Discussion

3.1. Sample

The samples mixed petroleum oil and soil as initial TPH concentration of samples. The measurements were done by solvent soxhlet extraction using n-hexane solvent with three repetitions; the average concentration of TPH was 18.64% [13].

3.2. Oil Recovery Test

Crude bio surfactant of each bacterium was applied to the soil samples with various concentrations molasses as carbon resources. Variations molasses carbon source provided on crude bio surfactant was 0, 5, 10, 15, 20 and 25 % for each of the bacteria and the mixture aerated for 10 days. The results of the acquisition and application of soil containing petroleum with crude bio surfactant TPH also be measured, as well as the measurement of the initial TPH. TPH measurement data used to calculate the recovery of petroleum for each bacterium. Petroleum recovery results using the bio surfactant of each bacterium can be seen in the figure below:

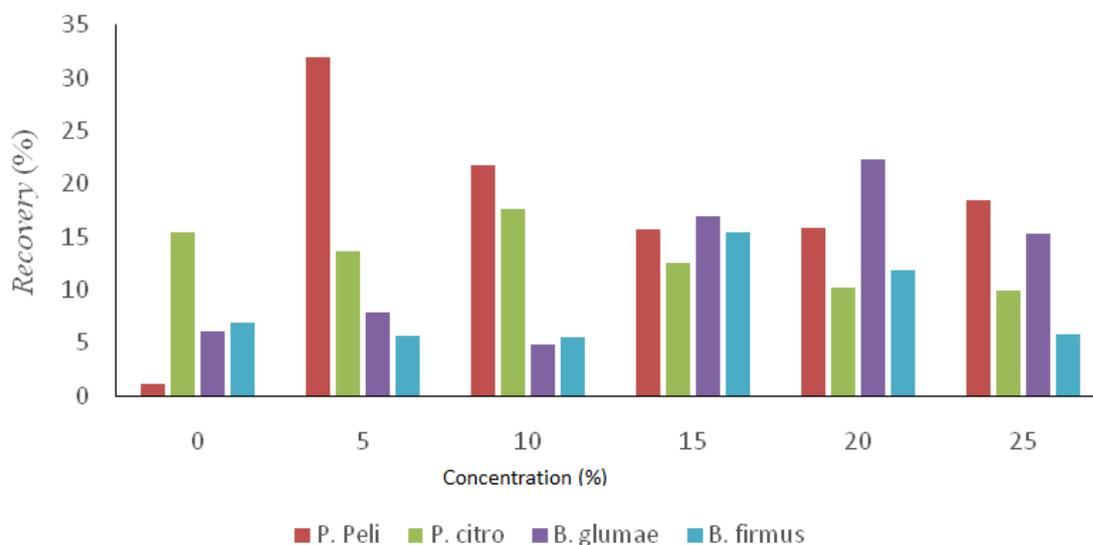


Figure 1. Oil recovery yield after the addition of molasses carbon source for each bacterium

Based on that data, four of these bacteria produce bio surfactant different, where the bacteria *Pseudomonas peli* bacteria produce oil recovery highest molasses carbon source 5%. This indicates that the *Pseudomonas peli* bacteria could effective recovery of petroleum. In this study, the number of medium Zobell added is constant, so it does not affect the performance of the bacteria because bacteria performance only influenced by the addition of molasses carbon source. The ability of the bacteria can be seen from the clear zone on an agar medium [9]. Each of these bacteria produces a clear zone different extent. Clear zone formed quite well above 20 mm, the wider clear zone produced, the greater the potential for bacteria to produce bio surfactant. In former the study showed that the clear zone formed on the bacteria *Pseudomonas peli* amounted to 25.79 mm. Based on the extensive clear zone are formed and the bacteria *Pseudomonas peli* is a potentially good bacteria to produce bio surfactant than other bacteria. Bio surfactant activity is closely associated with the ability of bacteria to consume the carbon source [14]. The use of carbon sources too little will make the substrate does not have sufficient contact with the cells, while the source of carbon is too much will make the cell has enough room to interact with the carbon

source, so the production process bio surfactant not be optimal [15]. In this study, *Pseudomonas peli* grown on molasses carbon source at a concentration of 5% has the greatest recovery value.

3.3. Chromatogram Analysis Results using GLC

GLC analysis provides information on the number of components of the oil before and after using crude bio surfactant with molasses carbon source concentration variations of each bacterium. Furthermore, the work of bacteria then identified by comparing identical compound fractions of each bacterium and is equipped with an abundance of compounds percentage between samples before and after treatment. The determination of percent identical compounds and those abundances can explain the carbon fraction degraded or dissolved. The chromatograms before and after treatments, and the component hydrocarbons percent abundance either dissolved or degraded through histogram described as follows:

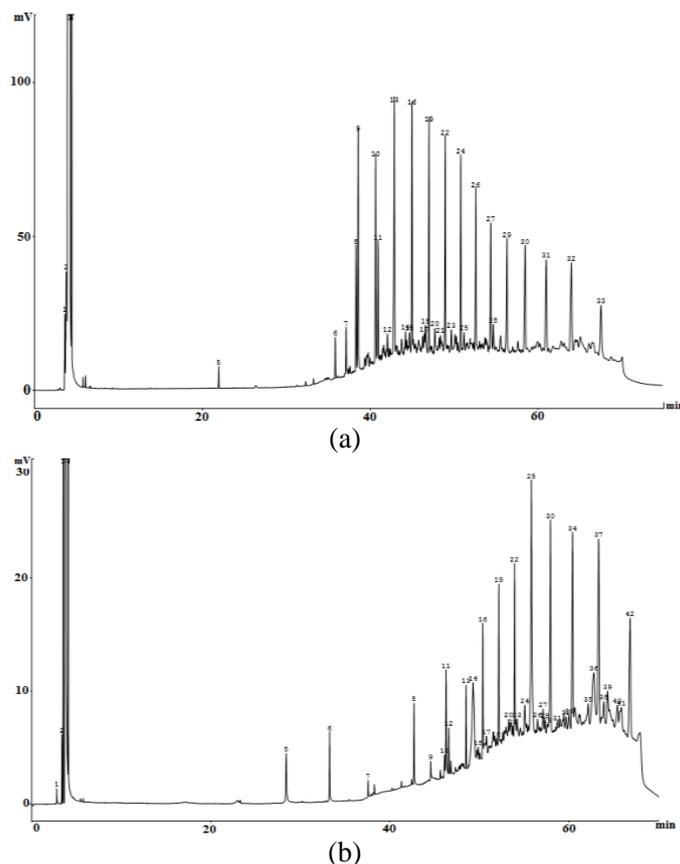


Figure 2. The chromatogram of initial condition sample (a), the chromatogram of sample after dissolved with bio surfactant of *P. peli* bacteria with carbon source concentrations of 5% molasses (b)

In the chromatogram of sample before treatment showed 33 peaks. Based on the total retention time for 67 minutes, the peaks of the chromatogram occurred at retention times 38.56; 40.65; 42.88; 44.99; 47.01; 48.94; 50.80; 52.58. After sample treated bio surfactant of *Pseudomonas peli* bacteria with the best concentration of carbon source molasses 5% have additional peaks from 33 to 42. Based on the total retention time for 67 minutes, there are six higher peaks in the chromatogram with retention time of 52.20; 53.96; 55.85 57.98; 60.45; 63.38 while the other had retention times of chromatogram with lower peaks

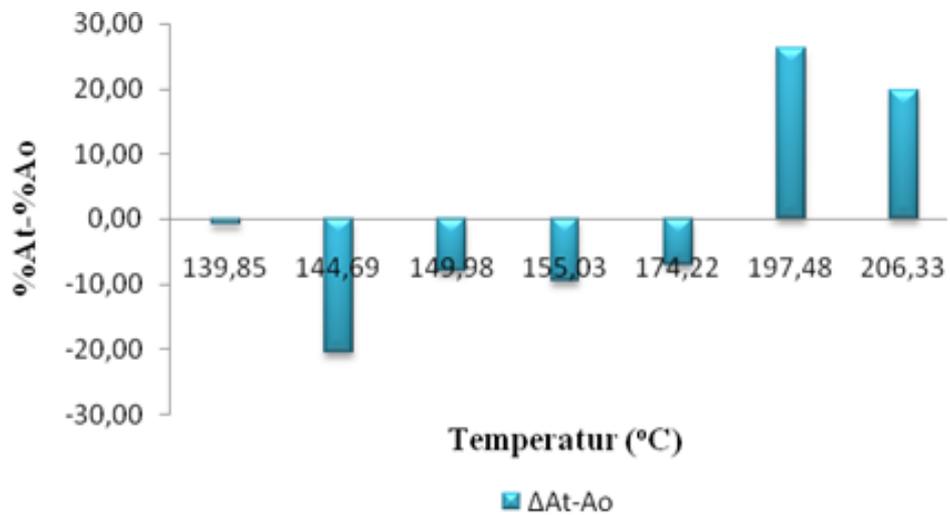
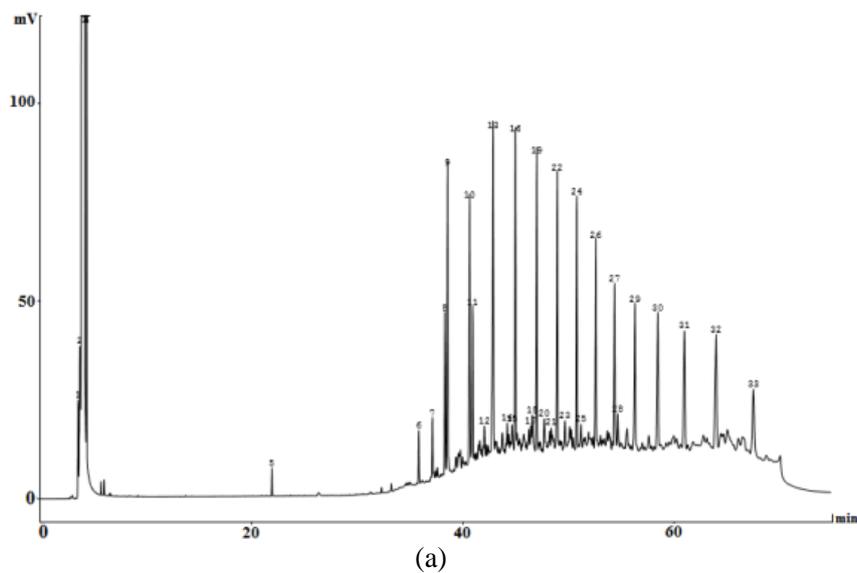


Figure 3. Histogram of dissolved hydrocarbons before and after the addition of crude bio surfactant *P. Peli* bacteria

Based on the above histogram, the difference in per cent calculated by subtracting the percent abundance peak after the addition of crude bio surfactant (At) of the *Pseudomonas peli* bacteria reduced percent of peak abundance prior to the addition of crude bio surfactant (A₀). The results were negative at baseline subtraction histogram shows that hydrocarbons short chain C atoms dissolved. Bio surfactant of *Pseudomonas peli* bacteria can dissolve petroleum compounds at temperatures fractions; 139.85; 144.69; 149.98; 155.03; 174.22°C. The highest temperatures dissolve compounds of hydrocarbons at 174.22°C.



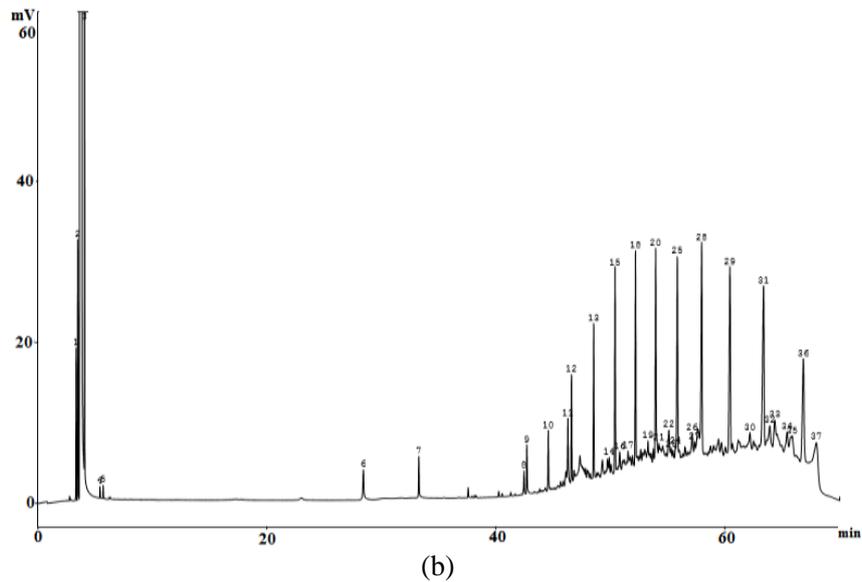


Figure 4. The chromatogram of initial condition sample (a), the chromatogram of sample after dissolved with bio surfactant of *P. citronellolis* bacteria with carbon source concentrations of 10% molasses (b)

Figure 4 shows the chromatogram of extracted sample by bio surfactant of *Pseudomonas citronellolis* bacteria with the best concentration of carbon source molasses 10% compared with the chromatogram constituents of petroleum before treatment have additional peaks from 33 to 37. Based on the total retention time for 68 minutes, there are eight highest peaks in the chromatogram with retention time; 48.54; 50.41; 52.20; 53.96; 55.85; 57.98; 60.44; 63.38 while the other had a retention time of chromatogram with lower peaks.

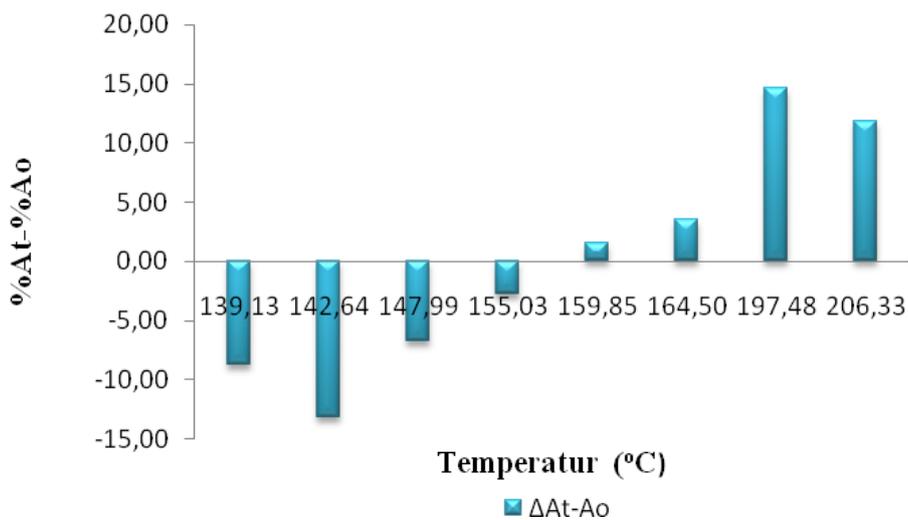


Figure 5. Histogram dissolved hydrocarbons before and after the addition of crude bio surfactant of *P. citronellolis* bacteria

Figure 5 shows that the hydrocarbon compounds with temperature fractions; 139.13; 142.64; 147.99; 155.03 °C decreased percent abundance. This suggests that the recovery of oil use bio surfactant of *Pseudomonas citronellolis* bacteria can dissolve compounds short hydrocarbon chains at temperatures fraction; 159.85; 164.50; 197.48; 206.33°C, and it shows that the long chain C atoms are degraded to form new compounds.

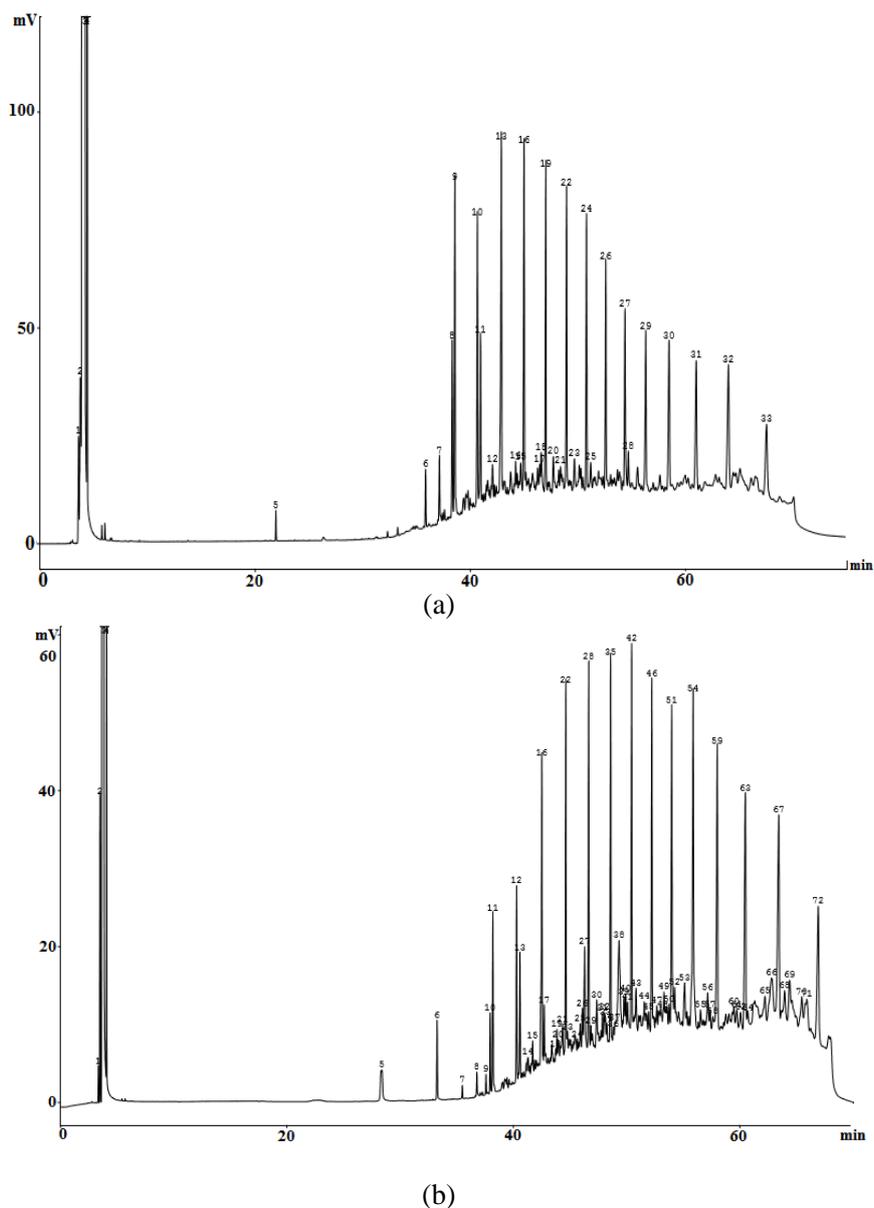


Figure 6. Chromatogram oil component (a), *B. glumae* chromatogram bacteria at concentrations 10% molasses (b)

Figure 6 shows the chromatogram oil component of the bacterium *Burkholderia glumae* with the best concentration of carbon source molasses 20% compared with the chromatogram constituents of petroleum

before treatment experienced a peak increase of 33 to 72 peak chromatogram peak. Based on the total retention time for 67 minutes, there were 11 chromatogram peaks higher than other peaks occur at retention times of 42.51; 44.63; 46.65; 48.59; 50.45; 52.23; 53.99; 55.88; 58.01; 60.49; 63.45.

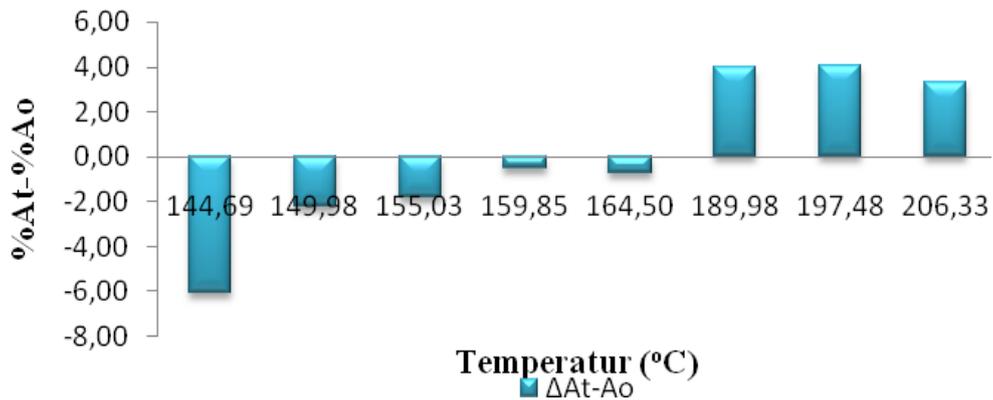
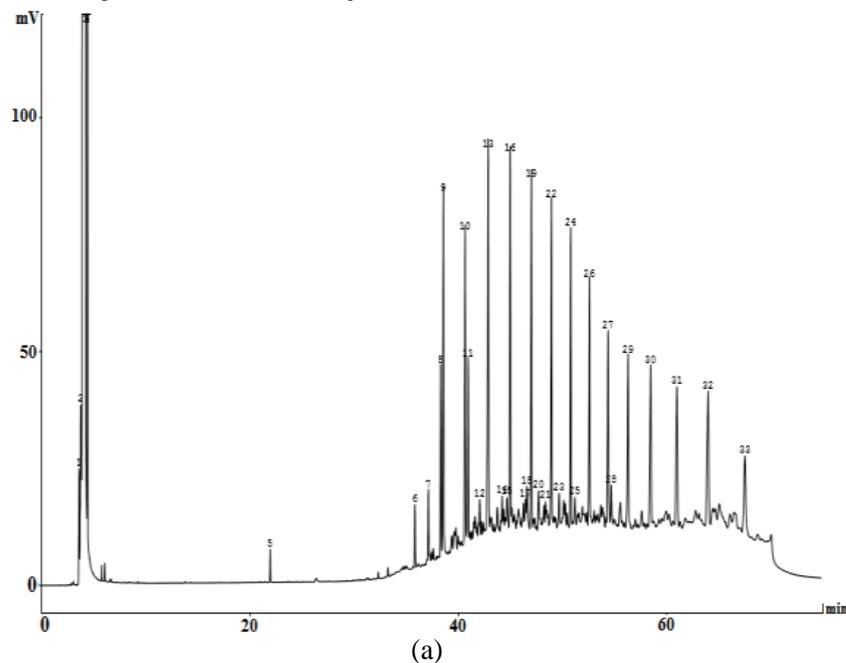


Figure 7. Histogram of dissolved hydrocarbons before and after the addition of crude bio surfactant of *B. Glumae* bacteria

Based on the above histogram, wide abundance peaks in early TPH before treatment with after the addition of crude bio surfactant of bacteria *Burkholderia glumae* is able to dissolve compounds C atoms shorter chain hydrocarbons at temperatures of 144.69; 149.98; 155.03; 159.85; 164.50 °C. In this bacterium maximum temperature to dissolve hydrocarbons short chain C atom is 164.50 °C



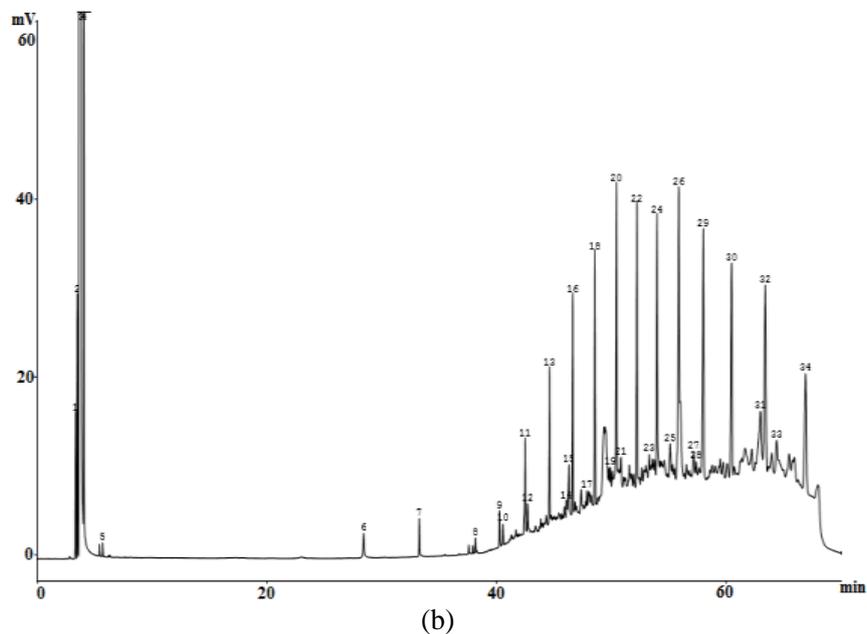


Figure 8. Chromatogram petroleum components, *Bacillus firmus* bacteria at concentrations of 15% molasses

Figure 8 shows the chromatogram oil component of the bacterium *Bacillus firmus* with the best concentration of carbon source molasses 15% compared with the chromatogram constituents of petroleum before treatment with bio surfactant of *Bacillus firmus*, the number peaks increase 33 to 34. Based on the total retention time for 67 minutes, there were 9 chromatogram peaks higher than other peaks occur at retention times; 46.63; 48.57; 50.43; 52.22; 53.98; 55.87; 58.01; 60.45; 63.42.

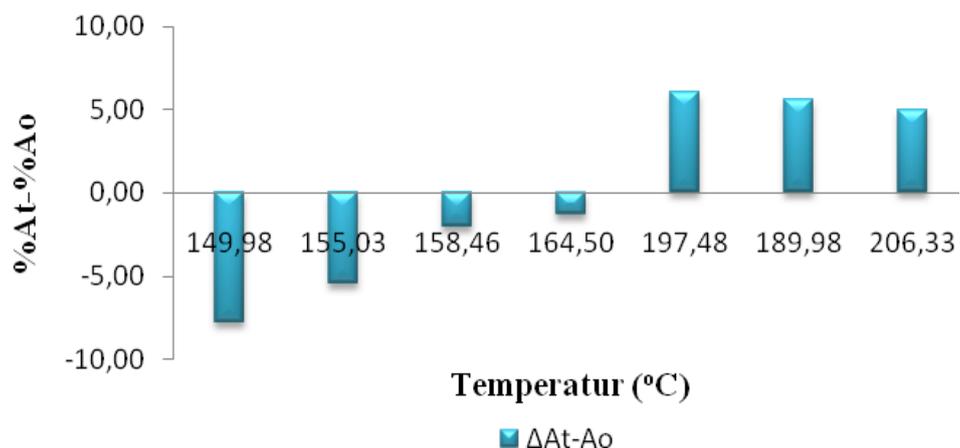


Figure 9. Histogram of dissolved hydrocarbons before and after the addition of crude bio surfactant of *Bacillus firmus* bacteria

Figure 9 shows that the hydrocarbon compounds with temperature fractions; 149.98; 155.03; 158.46; 164.50 °C decreased abundance percent or negative. This indicates that the recovery of petroleum using

bio surfactant from *Bacillus firmus* bacteria can dissolve hydrocarbons short chain C atoms. The highest temperature fraction dissolving the hydrocarbon short C chain is at 164.50°C. The fifth chromatogram shows that petroleum is a compound that is composed of many components of the hydrocarbon. Comparison of chromatograms of petroleum and recovery results with bio surfactant chromatogram of each bacterium has a change. Changes chromatogram peaks can occur because the process of degradation by bacteria. The results may indicate a degradation peaks are missing, emerging, increasing peak heights or lowering peak height. Lowering peaks height shows that the bacteria are able to degrade hydrocarbons perfectly. The bacteria degrade and break down long carbon chains into radicals, so that the amount of carbon captured GLC showed the presence of additional amounts of carbon and increasing the number of peak of the initial components [16]. Bacteria have the largest percentage recovery are different at each concentration of molasses. *Pseudomonas peli* produces the greatest recovery, namely the addition of molasses concentration as much as 5% in the amount of 31.92%, the bacteria *Pseudomonas citronellolis* produces the greatest recovery in the addition of molasses concentrations as much as 10% in the amount of 17.65%. Meanwhile, the *Burkholderia glumae* bacteria on the addition of molasses concentration of 20% with a recovery yield of 22.32%, and the bacterium *Bacillus firmus* on the addition of molasses concentration of 15% with a recovery yield of 14.38%. From the result of the recovery in each bacterium is a quantitative analysis, while for qualitative analysis is to analyze hydrocarbons dissolved and remains as a residue which is shown in the graph percent abundance. The fraction of carbon chains contained in the chromatogram can be identified based on the methodology temperature program [16].

4. Conclusions

Based on the research results can be concluded that:

- The optimal concentrations of molasses carbon source to produce bio surfactant effective for the recovery of oil is 5% molasses concentration to the *Pseudomonas peli* bacteria, molasses concentration of 10% for the *Pseudomonas citronellolis* bacteria, molasses concentration of 20% for the *Burkholderia glumae* bacteria, and molasses concentration of 15% for *Bacillus firmus* bacteria.
- The *Pseudomonas peli* bacteria with optimal concentrations of 5% molasses are the highest % recovery with a value of 31.92%.
- Based on the results of GLC analysis, the four bacteria are able to dissolve hydrocarbon compounds with C atoms of short chain hydrocarbons.

References

- [1] Juli N and Virmuda B 2001 Penelitian awal terhadap delapan isolat bakteri reservoir dalam mengembangkan volume minyak bumi secara monokultur *Prosiding Simposium Nasional IATMI* 2001 3-5 Oktober 2001 Yogyakarta
- [2] Baker C and Herson D 1994 *Bioremediation* (New York : Mc-Graw Hill, Inc.)
- [3] Karanth N G K, Deo P G, and Veenanadig N K 2005 Microbial Production of Biosurfactants and Their Importance (Indian Institute of Science) pp 1-18
- [4] Gudina E J, Jorge F B A, Ligia R R, Joao A P C, and Jose A T 2012 Isolation and Study of Microorganism from Oil Samples for Application in Microbial Enhanced Oil Recovery. *International Biodeterioration and Biodegradation* **68** 56-64
- [5] Desai J D and Banat I M 1997 Production of a Bio surfactant by *Pseudomonas fluorescens*. *Microbiol. Mol. Biol. Rev.* **61** 47-64
- [6] Laini R E, Napoleon A, and Munawar. Isolasi Bakteri Termofilik Penghasil Biosurfaktan yang Ber-potensi sebagai Agen MEOR (Microbial Enhanced Oil Recovery) dari Sumur Minyak di Sungai Angit *Jurnal Penelitian Sains* **17** 1-5
- [7] Zajic J E, Grignard H, Gerson F D 1977 Emulsifying and Surface Active Agents from

- Corybacterium hydrocarbonclatus*. *Biotech and Bioeng* **19** 1285-1301
- [8] Fajri C 2014 Test biosurfactants production by *Pseudomonas peli* on Different Carbon Sources as Agent Anti crust (scale inhibitor) to the Pipe Distribution of Petroleum (Indonesia : Dept.of Chemistry University of Sriwijaya South Sumatera).
- [9] Yudono B 2013 eksplorasi bakteri hidrokarbonoklastik dari rhizosfer di lahan tambang minyak rakyat, kecamatan babat toman, sumatera selatan *Jurnal Penelitian Sains* **13**
- [10] Sharpley J M 1996 *Elementary Petroleum Microbiology* (Texas : Gulf Publishing Company) pp 37 –109.
- [11] Munawaroh M 2014 Uji Recovery minyak dengan biosurfaktan dari bakteri yang toleran terhadap konsentrasi garam (Indonesia : Departemen of Chemistry, Mathematic and Natural Science Faculty of Sriwijaya University)
- [12] LECO Cooperation 2012 *Analysis of Light Crude Oil Using Gas Chromatography High Resolution Time of Flight Mass Spectrometry*. (Michigan USA : Saint Joseph.).
- [13] Agustin S 2014 *Biodegradasi minyak bumi dengan menggunakan bakteri indigen dari sumur tua desa babat toman musi banyuasin (MUBA)* (Indonesia : Dept.of Chemistry University of Sriwijaya South Sumatera)
- [14] Fiecter A 1992 Bio surfactant moving towards industrial application *Tibtech*. **10** 208-216
- [15] Fatimah 2007 Bio surfactants Production from *Pseudomonas sp* In Different Substrates *Berk. Penel Hayati* **12** 181-185
- [16] Yudono B 1994 *An Investigation Into The Prenature Cracking Asphaltic Pavement in Hot and Climated Thesis School of Chemistry* (England :University of Bristol)