Oil Recovery Tests by using Bio surfactant of Indigenous \textit{Pseudomonas peli} and \textit{Burkholderia glumae} Bacteria from South Sumatera at Various Temperature Conditions

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Abstract. The isolates of indigenous \textit{Pseudomonas peli} and \textit{Burkholderia glumae} bacteria were obtained from Babat Toman Village, Musi Banyuasin, South Sumatera which have a potential as bio surfactant. The bacteria are thermophile bacteria, and then they were used to produce bio surfactant by using molasses as carbon source. The bio surfactant were tested their ability to the oil recovery of soil contained oil 18.64%. The variables of treatments were temperatures from 40$^{o}$C to 80$^{o}$C. The results showed that the optimal oil recovery by using \textit{Pseudomonas peli} and \textit{Burkholderia glumae} were 2.20% and 18.19% at temperatures 80$^{o}$C and 40$^{o}$C respectively. The results of optimal oil recovery of each bacterium were analysed using gas chromatography to determine the constituent components of petroleum that can be extracted by using the bio surfactant. The bio surfactant of \textit{Burkholderia glumae} dissolved hydrocarbons at a fraction of temperatures 133; 139; 155; 156.8; 165; 173; 190; 197.5 and 206.3$^{o}$C with the total abundance of soluble oil 25.22%. Furthermore, the bio surfactant of \textit{Pseudomonas peli} dissolved hydrocarbons at a fraction of temperatures 133.3; 134; 139; 140; 145 and 150 $^{o}$C with the total abundance of soluble oil 16.94%.

Keywords: bio surfactant, temperature, oil recovery, \textit{Pseudomonas peli}, \textit{Burkholderia glumae}

1. Introduction

Microbial Enhance Oil Recovery (MEOR) is a technology that utilizes microbial activity by injecting microbes / bacteria into the reservoir to increase oil recovery. This technology is applied in tertiary recovery technology utilizing microorganisms and/or their metabolic products for recovery of residual oil [1]. The metabolic processes of bacteria can produce bio product such as bio surfactant, alcohol, biopolymers, gas, and acidity and can be proliferated if given the biocatalyst and appropriate nutrition. Bio product produced is able to extract the optimal oil recovery by improving displacement efficiency and reduce the viscosity of the oil trapped in the reservoir [2]. However, the use of chemical surfactant or physical processes such as pressurization, water flooding of steaming is generally inappropriate to most oil reservoirs [3]. The use of chemical surfactants for cleaning up oil reservoirs is not favorable in practice, because it is hazardous and costly and will leave undesirable residues which are difficult to dispose without adversely affecting the environment[4].
Low oil production in the tertiary phase because the remaining oil dominated by long chain hydrocarbon compounds, high viscosity and low mobility. High interfacial tensions between the water and oil may also produce high capillary forces retaining the oil in the reservoir rock [5]. Some bacteria may produce bio surfactants having two hydrophilic and hydrophobic active groups, for example *Pseudomonas sp*, *Bacillus sp*, and *Burkholderia sp* bacteria [6][1]. Bio surfactants have hydrophilic and hydrophobic groups so they can be partitioned between phases in water/oil and decrease the interfacial of surface tension [7]. Therefore, bio surfactant potentially can be used as Microbial Enhanced Oil Recovery (MEOR) [8][9]. Bio surfactant has several advantages over the chemical surfactant such as lower toxicity, higher degradability and better environmental compatibility, higher foaming, higher selectivity and specific activity at extreme pH, temperature and salinity [10].

The use of indigenous bacteria has the advantage of being adapted to the environmental conditions such as temperature, salinity, and pH. The *Pseudomonas peli* and *Bhurkholderia glumae* are thermo tolerant bacteria [11]. It is expected that the bio surfactant produced by the bacteria will also be suitable for the conditions.

This studies, isolation and identification of indigenous bacteria from South Sumatera which producing bio surfactant used to extract crude oil from soil contained oil at various temperature conditions (40-80°C). The bio-products excreted by these strains cause a series of very desirable changes in the properties of the crude oil [12].

2. Materials and Methods

Sterilization Equipment: All heat-resistant equipment sterilized by autoclave at a temperature of 121°C. Other tools sterilized with 70% alcohol.

2.1. Sampling

Samples were collected from 8 different areas of oil contaminated soils, extracted oil reservoirs, oil pipeline leakages and oil sewage and sludge dumps in Babat Toman Village, Banyuasin District of South Sumatera.

2.2. Cultivation media [13]

NA medium 150 mL inserted each 5 mL in a test tube. Tube containing NA tilted medium and allowed to stand for 24 hours. 1 Ose *Pseudomonas peli* inoculated on NA medium that has been hard and incubated at 37°C for 48 hours. The same treatment was done for *Bhurkholderia glumae* bacteria.

2.3. Regeneration Time of Bacteria [14]

Medium ZoBell as much as 100 mL plus 10 mL molasses autoclaved for ± 1 hour and cooled at room temperature ± 30°C. The medium was added 2 oses isolates of *Pseudomonas peli*. Bacterial inoculum solution was shook for 18 hours and every 3 hours a solution of 2 mL taken of and put into a test tube. Bacterial inoculum solution in a test tube which been separated from hour to 3, 6, 9, 12, 15, and 18 is inserted into the petri dish. Bacterial growth calculated using a counting chamber was placed on the microscope so that the resulting bacterial growth curve and found the shortest regeneration time of the bacteria. The same treatment as above was performed for both bacterial isolates that *Bhurkholderia glumae* so that the resulting curve obtained bacterial growth and shortest regeneration time. A generation time of bacteria could be calculated by the formula:

\[ g = \frac{2\log X_f}{(\log X_f - \log X_0)} \]
Where:
\[ X_f = \text{final number of cells (cell)} \]
\[ X_0 = \text{initial cell number (cell)} \]
\[ g = \text{regeneration time (cells / hour)} \]

2.4. Preparation of sample for oil recovery test
Soil was heated for 24 hours at a temperature of 110°C. Dry soil crushed using a mortar until smooth and screened so that the soil particles size 200 meshes. Sample soil prepared by 30 g of sludge oil and 70 g finely soil put into a glass beaker, and stirred until homogeneous.

2.5. Measurement of initial TPH of the sample [15]
Boiling flask of 250 mL size with soxhlet extraction tool which going to be used, dried in an oven, and cooled in a desiccator. A total of 10 g soil weighed in a filter paper. Filter paper containing the soil samples were placed in soxhlet extraction tool, further on it mounted condenser apparatus and below the boiling flask using n-hexane solvent by volume wetting the entire filter paper containing the soil samples and oil was then extracted using reflux. Reflux is performed until the solvent went back down to the boiling flask colorless. Solvent present in the boiling flask is distilled and collected, and then the boiling flask containing the extraction is heated in an oven at 100°C for one hour and then stored in a desiccator. After that, the flask along with the oil is weighed to constant weight. Percentage of Total Petroleum Hydrocarbons (TPH) petroleum is calculated using the formula:

\[
\%TPH = \frac{W_1 - W_2}{W_3} \times 100\%
\]

Where:
\[ W_1 = \text{weight boiling flask and extract petroleum (g)} \]
\[ W_2 = \text{weight of the empty boiling flask (g)} \]
\[ W_3 = \text{weight of the soil sample (g)} \]

2.6. Zobell liquid medium preparation [16]
Each ingredient is weighed according to the composition. All materials incorporated into the Erlenmeyer and dissolved by adding 1 L of distilled water and then inserted magnetic stirrer that serves to homogenize the solution and then covered with cotton and boiled using a hot plate. Erlenmeyer mouth covered with aluminum foil, and then sterilized in an autoclave for 20 minutes at a temperature of 121°C.

2.7. Starter Bacteria preparation [17]
Bacterial culture as much as 5 ose inoculated into each 100 mL of liquid medium and shaken ZoBell using a shaker for 24 hours at 120 rpm. Each subculture bacteria inoculated into Erlenmeyer containing 100 ml of liquid medium ZoBell based on the shortest regeneration time while still shaken using a shaker. *Pseudomonas peli* and *Bhurkholderia glumae* regeneration times are 12 hours.
2.8. **Crude bio surfactants production** [18]

Medium ZoBell as much as 100 mL mixed with 5 % (v/v) molasses into the flask. Media moved into Erlenmeyer, and then into the medium is inoculated 10 % starter bacteria. Culture aerated for 24 hours, and then incubated with a temperature of 40°C for 24 hours.

2.9. **Effect of Temperature on Oil Recovery** [19]

Erlenmeyer already containing 10 g of soil contaminants coupled with 200 mL of crude bio surfactant of *Pseudomonas peli* then aerated and heated to a temperature variation of 40, 50, 60, 70, 80°C for 7 days and then filtered using a filter paper. The resulting precipitate washed with distilled water and dried at a temperature of 100°C. The precipitate was dried and calculated so the percentage TPH. The same treatment is also performed using bacteria *Burkholderia glumae*.

2.10. **Initial components in sample analysis by using Gas Chromatography** [15]

Extract oil from sample diluted with n-hexane at a ratio of 1:9. The results of such dilution are injected into the GC apparatus with injector temperature of 290°C. Sample 2.5 μL is injected at 40°C on hold for 5 minutes, then the temperature is increased every minute to the temperature rise 5°C to 50°C, then the temperature was raised 10°C per minute up to 280°C and hold for 5 minutes. The mobile phase used is Helium. This procedure is also used to analyze of sample components after dissolved into crude bio surfactants.

3. **Results and discussion**

3.1. **Regeneration time Pseudomonas peli and Burkholderia glumae bacteria**

The growth curve was conducted to determine the optimum growth rate and optimum formation of metabolites produced by a microbe. The growth curve of bacteria was made with the intention to see the growth phases of the respective bacteria, namely the phase lag, exponential, stationary and death.

![Figure 1. Growth curve of bacteria *Pseudomonas peli*](image-url)
The shortest regeneration time of *P. peli* and *B. glumae* are 2.5 cells/hour and 2.35 cells/hour during 12 hours respectively. The shortest regeneration time is achieved when the growth rate rise sharply. This growth rate constant shows the number of bacteria that grow per unit of time in culture that is growing exponentially. Judging from both bacteria constant growth rate is highest in *B. glumae* i.e. $0.295 \text{ hour}^{-1}$, whereas *P. peli* growth rate constant was $0.277 \text{ h}^{-1}$. *P. peli* and *B. glumae* used in oil recovery treatment should be based on a growth curve. Both of these bacteria have the same exponential phase so in this study the bacteria are combined with each other. Thus, each of the bacteria will not have competition carbon source during its growth[20][7].

3.2. Measurement of Total Petroleum Hydrocarbons in initial sample

Measurement of Total Petroleum Hydrocarbon (TPH) is performed three repetitions on the soil sample is mixed with petroleum sludge generate initial TPH value by an average of 18.64 % from the three repetitions. Measurement of initial TPH samples land based on solubility in n- hexane extraction. After extraction the solvent is evaporated, subsequently determined by gravimetric TPH, it means physically separated analyses from the sample or from the solvent. Stages measurement initial TPH content of soil samples carried by solvent extraction using soxhletation. TPH is measured to determine the percentage of hydrocarbons in sample.

3.3. Effect of Temperature on the Recovery of Oil on Each Bacteria

Crude bio surfactant of *Pseudomonas peli* and *Burkholderia glumae* applied to oil recovery on soil contained petroleum with temperature variation of 40°C to 80°C with 5% molasses concentration and incubated for 7 days. Both of these bacteria are able to live in temperatures of 40°C to 90°C or in the grouping of thermophilic bacteria[18][12][5].

![Figure 2. Growth curve of bacteria *Burkholderia glumae*](image-url)
Based on Figure 3, *P. peli* shows that the higher temperature of crude bio surfactant, the percentage recovery of petroleum also increased. At temperatures of 40; 50; 60; 70; and 80 °C percentage recovery of petroleum from *P. peli* 0.16; 1.77; 2.15; 2.20; and 2.20 % respectively. However, bio surfactant produced by these bacteria is relatively small. Contrary, in Figure 4 *B. glumae* shows that the higher the temperature of crude bio surfactant, the percentage recovery of petroleum decreased. At a temperature of 40 ; 50 ; 60 ; 70 ; and 80 °C percentage recovery of oil from *B. glumae* i.e. 18.19 ; 15.45 ; 15.29 ; 14.97 ; and 14.81 % respectively. *B. glumae* produce bio surfactant relatively higher than the *P. peli*.

The results obtained in this study can be prove that the bio surfactant produced by *B. glumae* amounted to 81.25% and *P. peli* bio surfactant produced relatively little that is equal to 33.75% [3]. At different temperatures by both bacteria in producing petroleum recovery percentages because, each bacterium has a specific temperature to produce bio surfactant. The highest population of *P. peli* range of 2x10⁶ cells at 90°C, while *B. glume* highest population range 6 x 10⁶ at 40°C [3]. The ability of the bacteria can be seen from the clear zone on an agar medium[5][21][22]. The more extensive the resulting clear zone, the greater the potential for bacteria to produce bio surfactant. The results of the study, said that the clear zone produced by *P. peli* and *B. glumae* by 25.79 and 21.33 mm respectively. However, in this study *B. glumae* produce bio surfactant more than *P. peli*. This may be caused by the influence of temperature in the bio surfactant produced *P. peli* less than optimal. The ability of bacteria to produce bio surfactant relates to the ability of bacteria using hydrocarbons as substrates. Microorganisms with great bio surfactant generally have a large capacity also in solving hydrocarbons [21][23].
3.4. Analysis components of crude oil samples before and after treated crude bio surfactants at variation of temperatures

GC analysis provides information on the number of components making up the petroleum before and after treatment by using crude bio surfactant. GC analysis results are shown in Figure 5.
Figure 5. (a) The chromatogram of petroleum oil at initial condition, (b) Chromatogram of sample after treatment by using Pseudomonas peli, and (c) Chromatogram of sample after treated by using Bacteria Burkholde glumae.

Figure 5 shows 33 peaks of chromatogram of oil prior to treatment. Based on the total retention time for 67 minutes, the highest peak in the chromatogram retention time of 38.561; 40.652; 44.99; 47.011; 48.94; 50.799; 52.58 while the other had a retention time of chromatogram with low peak. Samples of soil and oil after the addition of bio surfactant of Pseudomonas peli with the best test temperature is 80°C has 22 peaks of chromatogram with retention time for 67 minutes. The highest peak of the chromatogram at the retention time of 44.988; 47.019; 48.96; 50.826; 52.616; 54.415; 56.347; 58.528; 61.057; 64.061 while the other had a retention time of chromatogram with low peak. For the next sample after the addition of bio surfactant of Burkholderia glumae, the best test temperature is 40°C has 43 peaks of chromatogram with retention time for 67 minutes. The peaks of chromatogram at the retention times of 33.305; 42.835; 46,434 have high peak while the others had a retention time of chromatogram with low peak. The third chromatogram shows that oil is a multi-component compound [9]. The performance of these two bacteria can be seen on the chromatograms of initial sample and after each treatment by comparing the percentage abundance of compounds between samples. Changes chromatogram peaks can occur because the process of degradation by bacteria. The results may indicate a degradation peaks are missing, emerging, growing peak or reduced peak. Solving hydrocarbon compounds into simpler compounds is largely determined by the structure of the hydrocarbon molecules and types of bacteria[4][14][23].

Degraded hydrocarbon compounds can be demonstrated by comparing the data histogram with peaks identical carbon fraction of percent abundance of each component. Of the three, the chromatogram peaks there are some areas that have a fraction of the carbon chromatogram peaks were identical. The carbon chains are identical peak on the chromatogram TPH before the addition of crude bio surfactant and chromatogram TPH after the addition of crude bio surfactant. The determination of peak abundance percent identical and can be describes the fraction of carbon degraded or dissolved [24].
The difference in percent abundance of component is calculated by subtracting the percent abundance peak after the addition of crude bio surfactant ($A_t$) from the bacterium *Burkholderia glumae* reduced percent of peak abundance prior to the addition of crude bio surfactant ($A_0$) as shown in Figure 6. From the results of these reductions was found that hydrocarbon compounds are dissolved in bio surfactant of bacteria *Burkholderia glumae* obtained if the result of subtraction is negative, which means that the hydrocarbon compounds soluble bio surfactant[25]. Histogram declining indicates that the short-chain hydrocarbon compounds soluble bio surfactant, so that the chromatogram peaks are short carbon chain is missing. Such changes occur at a temperature of 133; 139; 155; 156.8; 165; 173; 190; 197.5 and 206.3 °C, where the short-chain hydrocarbons dissolved in abundance percentage of 25.22%. Long-chain hydrocarbons increase in temperatures program 145; 149; 153.5 and 154 °C.

![Figure 6](image)

**Figure 6.** Changes in % Abundance After treatment with the *B. glumae* bacteria

In Figure 7. The components with temperatures of 133.3; 134; 139; 140; 145 and 150 °C are decreased in percent abundance of components. This indicates that the bio surfactant of *Pseudomonas peli* bacteria can dissolve petroleum hydrocarbons with an abundance percentage of 16.94 %. At temperatures of 160 ; 165 ; 169; 173 ; 174 ; 178 ; 184 ; 190 ; 197 and 206.5 °C percent abundance

![Figure 7](image)

**Figure 7.** Changes % Abundance After treatment with the *P. peli* bacteria
increased. These circumstances show that the long-chain hydrocarbon compounds remaining in the residue and insoluble in bio surfactant. Meanwhile, if there is a decrease the microbial hydrocarbon is used as an energy source, or dissolved this has led to wide differences in each area at the same retention time[26].

4. Conclusion

Based on this study, we can conclude:

1) The temperature that produces% recovery of oil in *Burkholderia glumae* maximum occurs at a temperature of 40°C by 18.19% compared to the relatively higher than *Pseudomonas peli* occurs at a temperature of 80°C equal to 2.20%.

2) The bacterium *Burkholderia glumae* bio surfactants can dissolve hydrocarbons of petroleum fractions temperatures 133; 139; 155; 156.8; 165; 173; 190; 197.5 and 206.3 °C with total abundance of 25.22%, while the bio surfactant produced by *Pseudomonas peli* can dissolve hydrocarbons of petroleum fractions temperature 133.3; 134; 139; 140; 145 and 150 °C with a total abundance of 16.94%.

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