Kinetics of Petroleum Oil Recovery in Bio Surfactant of *Brevundimonas diminuta* and *Pseudomonas citronellolis* Bacteria Solutions

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Keywords: Bio Surfactant, *Brevundimonas diminuta*, Kinetics, *Pseudomonas citronellolis*.

Abstract: Kinetics of crude oil solubility in bio surfactant of *Brevundimonas diminuta* and *Pseudomonas citronellolis* bacterias was studied by using a combination of differential and integral methods. The order of reaction and reaction rate constants of crude oil solubility was determined by using differential and integral methods respectively. The observation was carried out for 10 days, and integral method observation was carried out every 2 days, for 10 days. The initial TPH concentration (Total Petroleum Hydrocarbon) of sludge sequently were 1.64; 3.28; 4.91; 6.11 and 7.57 % (w/w). After 10 days' treatment by using *B. diminuta* bacteria, the TPH concentrations were decreased to 1.24; 2.43; 3.61; 4.32; 5.07% (w/w) and the TPH concentration using *P. citronellolis* decreased to 1.07; 2.10; 3.13; 3.79; 4.40% (w/w). The order reaction which determined by using differential method were 1.81 for *B. diminuta* and 1.1 for *P. citronellolis*. Furthermore, the order reaction was put in the integral equation thus forming reaction rate constants equation of crude oil solubility which are 0.018 day^{-1} for *B. diminuta* and 0.033 day^{-1} for *P. citronellolis*. Qualitative analysis which were carried out by using GC showed that bio surfactant from *B. diminuta* was able to dissolve hydrocarbon compounds of crude at fraction with lower C atomic chain <C10–C14 and C atomic longer >C22. Bio surfactant from *P. citronellolis* was able to dissolve hydrocarbon compound with atomic chain C10 – C14.

1 INTRODUCTION

Petroleum is a very strategic natural resource to increase the source of foreign exchange for the country. This type of natural resource is classified as non-renewable and its management must be carried out very effectively and efficiently. Since the last decade, the exploitation of oil fields in Indonesia has not found a significant number of new reservoirs to maintain and increase oil production. Meanwhile, the role of petroleum has yet to be replaced even though the search for petroleum deposits has become increasingly difficult, which affected its exploitation in an increasingly expensive manner (Juli and Virmuda, 2001).

The process of exploitation or recovery of petroleum in primary recovery method can recover 30-40% of petroleum, and secondary recovery that can recover about 15-25% of petroleum. From this method, it turns out that it still leaves around 60-70% of the oil content trapped in the earth's crust (Geetha, Banat and Joshi, 2018; Al-Bemani, 2011).

Given that the releasable oil trapped in rocks is still quite high, which is around 60-70%. One of the technologies to increase the acquisition of petroleum is by using microorganisms known as the Microbial Enhanced Oil Recovery (MEOR) method (Omoniyi, 2015; Nai, Magrini and Offe, 2016).

Isolating microbial indigen from Babat Toman village which has the potential to produce bio surfactants including *P. acidovorans*, *Brevundimonas diminuta*, *P. flourescens*, *Bukholderia glumae*, *P. aeruginosa*, *Bacillus firmus*, *P. peli*, and *P. citronellolis*. In this study, *Br. diminuta* and *P. citronellolis* bacteria are used. The effect of salt on oil recovery oil, where the study

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showed optimal salt for *B. diminuta* bacteria was reduced by 3% and *P. citronellolis* 6%. *B. diminuta* and *P. citronellolis* bacteria were used because these bacteria are able to produce bio surfactants and recover petroleum. This is the basis of this research, which needs further research to determine the reduction in the concentration of Total Petroleum Hydrocarbons (TPH) per unit time from the bacteria *B. diminuta* and *P. citronellolis*. Kinetics information is very important because kinetics provides information on chemical concentrations that are still lagging at any time and can be used to predict the length of time the oil degradation process will lead to the expected residual target (Marshall, 2008; Yudono et al., 2017).

In this study a sample concentration variation was carried out with a treatment time of 10 days. All data were evaluated using chemical kinetic theory with differential and integral method approaches. Qualitative studies were carried out using Gas Chromatography (GC) which gave a more detailed picture of the hydrocarbon compound fractions before treatment and after treatment (Jovančićević et al., 2004; Salminen et al., 2004).

2 MATERIALS AND METHODS

The material used in this study, namely microbial isolates *B. diminuta* and *P. Citronellolis* (obtained from the Microbiology Laboratory of the Biology Department of FMIPA Sriwijaya University), NA medium, aquadest, K_2HPO_4, peptone, $FeSO_4$, yeast extract, 70% alcohol, methanol, n-hexane, molasses (taken from sugar waste from PT Cinta Manis, Tanjung Raja, Ogan Ilir, South Sumatra) and oil sludge obtained from Babat Toman Village, Musi Banyuasin Regency.

2.1 Sterilization Tools

All heat-resistant equipment that will be used is sterilized using an autoclave with a temperature of 121ºC for 15 minutes, while non-heat-resistant equipment is sterilized using 70% alcohol.

2.2 Regeneration of Bacteria

Each bacterium of *B. diminuta* and *P. citronellolis* inoculated into NA solid media with zigzag movements. Previously NA solid media was sterilized in an autoclave at 121ºC. Bacteria that have been put into the NA medium are incubated in an incubator for 24 hours. After incubation the bacteria are ready to be used (Yudono et al., 2010).

2.3 Zobell Medium

The Zobell medium was made by dissolving 5 g of peptone, 1 g of yeast extract, 0.012 g of K_2HPO_4, and 0.01 g of $FeSO_4$ in distilled water with a solution volume of 1000 mL. The mixture is boiled on the hotplate and homogenized by stirring by a magnetic stirrer. After boiling the mixture is sterilized by autoclaving at 121ºC for 15 minutes (Yudono and Estuningsih, 2013).

2.4 Indigenous Bacterial Starters

The bacterial cultures of *B. diminuta* and *P. citronellolis* as many as 5 ose were subcultured by being put into each Erlenmeyer containing 100 mL of Zobell medium, then aerated for 24 hours. The 100 mL Zobell medium is added to the mixture until the total volume is 200 mL and the mixture is re-aerated until the shortest generation time. The shortest generation time of *B. diminuta* 12 hours and *P. citronellolis* 9 hours (Young et al., 2005; Yudono et al., 2011).

2.5 Crude Bio Surfactant Production

The total volume for bio surfactant production is 100 mL by mixing a bacterial starter, Zobell medium and molasses concentration (taken from the sugar waste of PT Cinta Manis, Tanjung Raja, Ogan Ilir, South Sumatra) with a concentration of 15% molasses for *B. diminuta* and 10% for the bacterium *P. citronilolis*) with various volume variations. Then the mixture is incubated based on the shortest generation time. The shortest generation time of *B. diminuta* is 12 hours and *P. citronellolis* 9 hours (Yudono et al., 2010).

2.6 Initial TPH measurement

A total of 10 g of sludge is inserted using filter paper that fits the size. The filter paper containing the sludge sample is then inserted into the soxhlet tube. Then the upper part of the soxhlet tube is connected to the condenser and the lower part is connected to the boiling flask containing n-hexane solvent with the volume soaking the entire filter paper. Soxhlet is done until the solvent drops back to boiling flask. Soxhletion is stopped when the solvent is clear on the soxhlet tube. Boiling flask which contains extracts is then heated in an oven at 100ºC, then

stored in a desiccator. Flask and oil are then weighed. Then the residue from soxhletion is weighed and the percentage of petroleum TPH is calculated using the equation:

$$\% \text{ TPH} = (W_1\text{-}W_2) / W_2 \times 100\% \quad (1)$$

Where: W_1 = Sludge sample weight (g), W_2 = Residual weight (g)

2.7 GC Analysis

Petroleum filtrate obtained from the soxhletion process is diluted with n-hexane 50 mL. The results of the dilution are taken several mL, to be injected on a Thermo Scientific GC device with a programmed temperature of 40°C maintained for 4 minutes, the temperature is raised 5°C per minute until the temperature reaches 300°C. The type of column used is TG-5MS with a column length of 30 m and a diameter of 0.25 mm.

2.8 Reaction Order of Petroleum Solubility

A total of 10 g sludge with a variation of TPH 1.5, 3.0, 4.5, 6.0, and 7.5% mixed with a bio surfactant mixture: distilled water 10% (v/v) to a volume of 100 mL. The mixture is then aerated for 10 days. The residue from the screening results was calculated as the percentage of TPH. The percentage of petroleum TPH is calculated using equation 2, while the filtrate is extracted with n-hexane 25 mL using a separating funnel. The top layer is taken by repetition 4 times, then analysed using GC.

2.9 Oil Recovery Rate Constant

A total of 10 g sludge with a 4.5% TPH concentration was added to each bio surfactant mixture: distilled water to a total volume of 100 mL. The mixture is then aerated according to the variation time, for 2, 4, 6, 8 and 10 days. The residue from the screening results was calculated as the percentage of TPH. The percentage of petroleum TPH is calculated using equation 1.

2.10 Determination of Reaction Order

Determining the value of the degradation reaction order (n) through the differential method, obtained directly from the graph of the ln [C] relationship with ln r as shown in equation 3, where ln [C] is TPH day 0 and ln r is TPH day 10 with the percentage of TPH varying 1, 5%, 3%, 4.5%, 6% and 7.5%, by determining the differential equation tangent.

$$r = \frac{-d[C]}{dt} = k[C]^n \quad (2)$$

$$r = \frac{d[C]}{dt} = -k[C]^n \quad (3)$$

$$\ln r = \ln k + n \ln [C] \quad (4)$$

Thus, the order of degradation reaction (n) is the same as the slope and can be directly determined after the incubation period of day 0 to day 10, the data is analysed using the integral and special method at a concentration of 4.5% by measuring TPH every 2 days. The integral method is done to determine the degradation reaction constant (k), based on the order of the degradation reaction (n), which has been determined from the differential method. Through the integration equation continuously calculated according to order (n), a graph of relationship time (days) was obtained with [TPH] (Yudono, 1994).

3 RESULTS AND DISCUSSION

Determination of the reaction order of TPH due to petroleum solubility is using differential methods. The reaction order is determined by graphing the relationship between ln [C] and ln r so that the differential equation can be determined (Marshall, 2008). Based on the equation (4), ln r = ln k + ln [C], if a graph is made of the relationship between ln [C] and ln r, then a straight line graph will be obtained with n being the slope and ln k being intercept. The reaction constant will be more accurate when measured using the integration method (Yudono et al., 2011). The Table 1 shows the decreasing TPH concentrations from day 0 to day 10 using bio surfactant of *B. diminuta* bacteria.

3.1 Phytochemical Screening

Important phytochemicals, such as alkaloids, triterpenoids, steroids, phenolics, flavonoids and saponins for their presence and are presented in Table 1.

The Table 2 shows the decreasing TPH concentrations from day 0 to day 10 using bio surfactant of *P. citronellolis* bacteria.

Table 1: Kinetics of solubility of petroleum sludge in bio surfactant of B. diminuta bacteria.

TPH sludge concentration (%)		Δ[TPH]	r	ln r	ln C_o
C_o	C_{10}				
1.64	1.08	0.56	0.056	-2.76	0.49
3.28	2.10	1.18	0.118	-2.09	1.18
4.91	3.13	1.77	0.177	-1.66	1.59
6.11	3.79	2.31	0.231	-1.32	1.81
7.57	4.41	3.16	0.316	-1.08	2.02

Table 2: Kinetics of solubility of petroleum sludge in bio surfactant of *P. citronellolis* bacteria.

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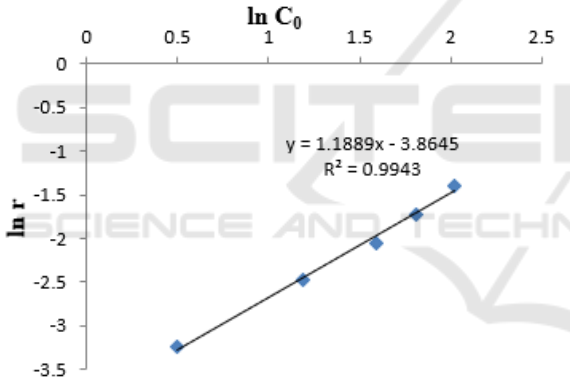

Figure 1: Graph of the relationship ln [C0] with ln r for the *P. citronellolis* bacteria.

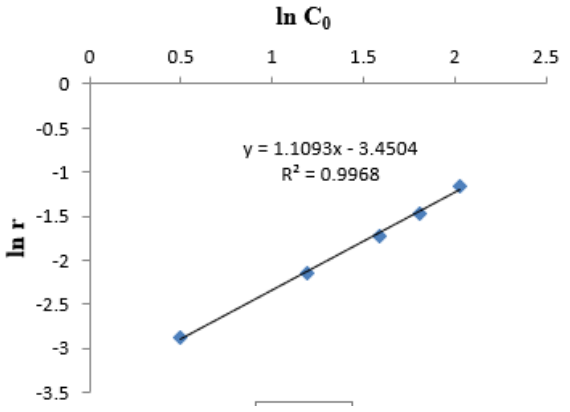

Figure 2: Graph of the relationship ln [C0] with ln r for the *P. citronellolis* bacteria.

From the Figure 1, a graph of the relationship ln [C_0] with ln r for *B. diminuta* bacteria obtained regression value obtained at 0.9943. This shows the graph is a straight line. From the results of the calculation of the straight-line equation, the slope (slope) is obtained by 1.18 with the intercept value of -3.86. According to the differential method, the slope value shows the order of the degradation reaction (n), so based on this value, the order of reaction for petroleum solubility using the bacterium *B. diminuta* is a reaction order 1.18. Usually the oil degradation reaction refers to first order reaction (Yudono, 1994); while in this study the reaction order is 1.18. For Figure 2, a graph of the ln [C_0] relationship with ln r *P. citronellolis* bacteria obtained a slope of 1.1 with an intercept value of -3.45. And the regression value is 0.9968. Based on these values, it can be concluded that, the order of reaction of petroleum solubility using *P. citronellolis* is a reaction order 1.1.

3.2 Determination of Petroleum Solubility Constants with Integral Methods

The reaction rate constant of petroleum solubility can be determined using the integral method. The integral method shows that the value of the slope is the value of the reaction rate constant of petroleum solubility. The kinetics data can be seen in Table 3 and 4.

Table 3: The kinetics data of petroleum sludge solubility in bio surfactant of *B. diminuta* bacteria.

Time (t)=x	Concentration (C_t)=y	ln [TPH]	$[Ct]^{-0.18}$
2	4.35	1.47	0.933
4	4.21	1.43	0.936
6	3.95	1.37	0.944
8	3.73	1.31	0.951
10	3.51	1.25	0.959

Table 4: The kinetics data of petroleum sludge solubility in bio surfactant of *P. citronellolis*.

Time (t)=x	Concentration (C_t)=y	ln [TPH]	$[Ct]^{-0.18}$
2	4.28	1.45	0.963
4	4.07	1.40	0.966
6	3.73	1.31	0.972
8	3.39	1.22	0.980
10	3.03	1.10	0.989

From the data table above each can be graphed $[Ct]^{-0.18}$ relationship with time (days), so that the slope and intercept values are known. Following is the graph of the relationship $[Ct]^{-0.18}$ with time (days):

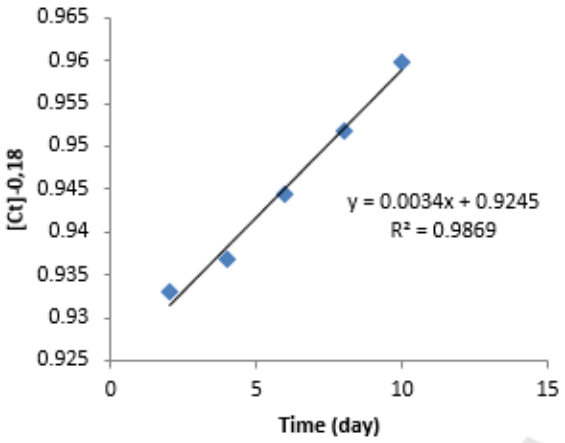

Figure 3: Graph of correlations of time (days) with concentrations $[C]^{-0.18}$.

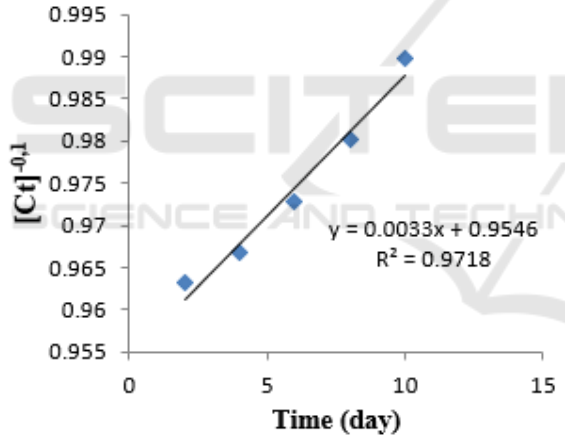

Figure 4: Graph of correlations of time (days) with concentrations $[C]^{-0.1}$.

Figure 4. Graph of the relationship of time (days) with $[C]^{-0.18}$ shows the line equation Y = 0.0034x + 0.9245. The slope value obtained is 0.0069 and intercept 0.9546. The regression value is 0.9869. This shows the graph is a straight line. Then Figure 4. Graph of relations of time (days) with $[C]^{-0.1}$ shows the equation of line Y = 0.0033x + 0.9546. The slope value obtained is 0.0033 with a regression value of 0.9718.

To determine the reaction rate constant, the slope values obtained from Figure 3 and 4 are inserted in equation 7.

$$\frac{-d[C]}{dt} = kC^n \quad (5)$$

$$\int_{C_o}^{C_t}[C]^n d[C] = -k\int dt \quad (6)$$

$$C_t^{-n+1} = C_t^{-n+1} - (n+1)kt \quad (7)$$

So that the constant value for *B. diminuta* bacteria is 0.0189 $days^{-1}$ and *P. citronellolis* is 0.033 $days^{-1}$. From Figures 1, 2, 3 and 4 we can see the comparison of each bacterium shown in Table 5.

Table 5: Order and constant of each bacterium.

Bacteria	Parameter	
	n	K (day^{-1})
B. diminuta	1.18	0.018
P. citronellolis	1.1	0.033

3.3 GC Chromatogram Analysis

The results of extracting petroleum sludge from petroleum sludge in the initial conditions and after extracting it with bio-surfactant were analyzed using GC. These results were then analyzed qualitatively using Thermo Scientific GC-MS to determine the difference in the number of sludge components before treatment with the number of sludge components after the addition of bio surfactant treatment from each bacterium (Jovančićević et al., 2004). Based on the GC-MS data that has been obtained, it can also be qualitatively analyzed the dissolved and non-dissolved hydrocarbons left on the residue shown in the histogram in the form of percent abundance of each bacteria.

Components that are dissolved in bio surfactants and components that become residues can be explained through histograms of changes in abundance. Based on the histogram, the x axis is the C chain fraction identified which is determined by determining the temperature range calculated from the retention time and the y axis is the change in abundance. The histogram was generated from the acquisition of the initial component chromatogram data and after the addition of bio surfactants from each bacterium. The abundance change is indicated by $\Delta A = \%A_t - \%A_o$, if the resulting data is a positive number, it shows the component of the dissolved hydrocarbon chain. Otherwise, if it is a negative number, it indicates that the hydrocarbon chain component is residual.

The fractions of C which are residues and dissolved ones can be seen in Table 6 which shows the C chain fraction based on its temperature (Fanani, Yudono and Situmorang, 2014).

Table 6: Hydrocarbon chain fractions based on temperature.

Temperature range (°C)	Carbon chain fraction
<100	$<C_{10}$
100-150	C_{11}-C_{14}
150-200	C_{15}-C_{17}
200-250	C_{18}-C_{21}
250-300	$>C_{22}$

Based on Figure 8a, it can be seen that there is a significant number of chromatogram peaks of petroleum constituent compounds in initial condition.

Based on the total retention time for 56 minutes, there were some of the highest peak chromatograms, which occurred at retention times 28.11; 33.26; 36.21; 38.74; 44.88; and 53.52, whereas for other retention times it has a chromatogram with a low peak. In Figure 4b chromatogram, it can be seen in the chromatogram that small peaks appear which indicate the presence of new compounds at the retention time of 6.34 which was previously not detected in Image Chromatogram 4a. After the addition of bio surfactants from the bacterium *B. diminuta*, Figure 4b shows a decrease and addition of the chromatogram peak with a total retention time of 56 minutes. In addition, Figure 4c also shows that small peaks have appeared at retention times of 5.13 which indicate the presence of new compounds. These compounds have low molecular weight because they appear at earlier retention times and are branching alkane isomers. There are several highest peak chromatograms, namely at retention time of 6.32; 9,79; 18.07; 33.65; 40.19, while for other retention times have a chromatogram with a low peak. From the picture above it can be concluded that there is a change in the rise and fall of the peak area in the chromatogram, whether it increases or decreases. Changes in the rise and fall of the peak area indicates that bio surfactants work to dissolve the hydrocarbon components to form new, simpler hydrocarbon components (Fanani, Yudono and Situmorang, 2014).

The component changes that occurred can be explained qualitatively on the changes in abundance shown in Figure 5 in the form of a histogram.

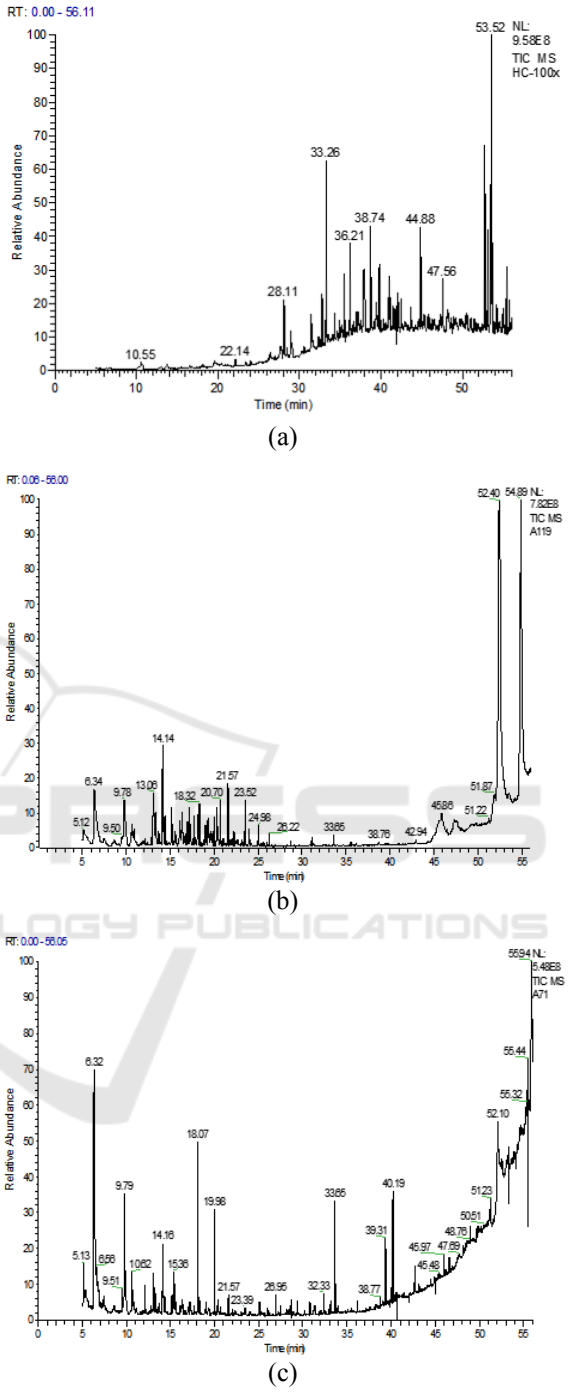

Figure 4: (a) Chromatogram TPH initial sludge before crude bio surfactant treatment, (b) Chromatogram TPH end sludge after treatment using bio surfactant from bacteria *Brevundimonas diminuta*, (c) Chromatogram TPH end sludge after treatment using bio surfactant from *Pseudomonas citronellolis* bacteria.

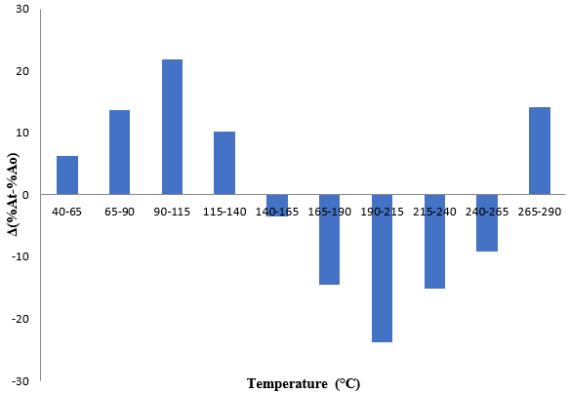

Figure 5: Histogram of changes in soluble oil abundance in bio surfactants of bacteria *Brevundimonas diminuta.*

Based on the histogram above, the percentage difference in abundance is calculated by subtracting the percentage of abundance after the addition of crude bio surfactant (A_t) from the bacterium *B. diminuta* minus the peak abundance percentage before the addition of crude bio surfactant (A_0). The positive reduction results show that dissolved hydrocarbons in bio surfactant. Increased histogram shows that short chain hydrocarbons dissolve in bio surfactants, so that at the peak of the chromatogram there are carbon chains that are lost and decompose into short chains. This histogram explained that bio surfactants from *B. diminuta* bacteria at a 5-20 minute retention time showed a percentage increase in abundance compared to the initial one, indicating that bio surfactants were able to dissolve short-chain C hydrocarbons namely atomic chains $<C_{10} – C_{14}$ at temperatures 40-140°C and at retention times 50-55 minutes are able to dissolve long C hydrocarbons, namely the atomic chain $>C_{22}$ at a temperature of 265-290°C which is at a positive value. But bio surfactants from *B. diminuta* bacteria at a retention time of 25-50 minutes were unable to dissolve long C hydrocarbons, namely C_{15}-C_{17} and C_{18}-C_{21} at temperatures of 140-265°C. While the component changes that occur in the use of *P. citronellolis* bacteria can be seen in Figure 6.

Based on the Figure 6, the increasing histogram shows that short-chain hydrocarbons dissolve in bio surfactants, so that at the peak of the chromatogram there are long carbon chains that are lost and break down into short carbon chains. the per cent difference in abundance is calculated by subtracting the per cent peak abundance after the addition of crude bio surfactant (A_t) from the bacterium *P. citronellolis* minus the percent peak abundance before the addition of crude bio surfactant (A_0).

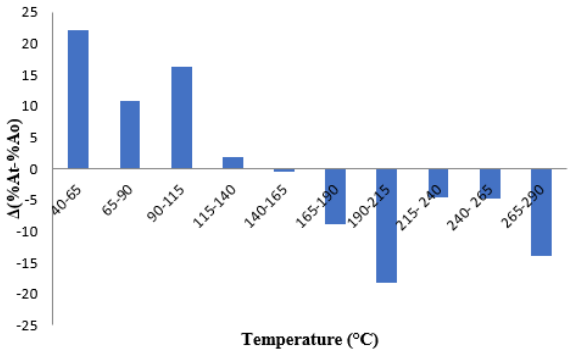

Figure 6: Histogram of changes in soluble oil abundance in bio surfactants of *Pseudomonas citronellolis* bacteria.

If the result of the reduction is positive, it means that the hydrocarbon compound dissolves in bio surfactant, where if bio surfactant and bacterial culture have a larger hydrophobic head then it will also produce a large oil recovery. On the histogram, it can be seen that most of the atomic chain hydrocarbons $<C_{10}$ - C_{14} are in a positive value, which means that the atomic chain hydrocarbons $<C_{10}$ - C_{14} are mostly soluble in bio surfactants.

4 CONCLUSIONS

B. diminuta and *P. citronellolis* bacteria can reduce the Total Petroleum Hydrocarbons (TPH) in all treatment media concentrations. The optimal reduction in TPH using bio surfactant from *B. diminuta* bacteria occurred at variations in media concentration of 7.51% (b / b) for 10 days with TPH values falling to 5.07% (b / b), while for *P. citronellolis* bacteria able to reduce TPH 7.51% to 4.40% within 10 days.

The reaction order and reaction rate constants obtained from petroleum solubility using the bio surfactant of the *B. diminuta* bacteria are 1.18 and 0.0189 days^{-1}. Based on the kinetics model, the time needed to dissolve petroleum sludge completely is in 73 days, while the reaction order and reaction rate constants of *P. citronellolis* are 1.1 and 0.033. the time needed to dissolve petroleum sludge completely is in 44 days.

The results of GC and Histogram analysis of changes in oil abundance from each bacterium can be seen that bio surfactants from *B. diminuta* bacteria based on 5-20 minutes retention time appear short-chain C hydrocarbons which are < C10 – C14 atomic chains obtained from long chain hydrocarbon degradation and at retention time of 50-55 minutes, it is able to dissolve long chain C hydrocarbons

namely atomic chain> C22, while bio surfactant from *P. citronellolis* bacteria based on 5-20 minute retention time can be seen dissolving hydrocarbon C atomic chain <C10-C14 which is the result of hydrocarbon decomposition long chains that can be seen from the decrease in the area of peak abundance.

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