Oil Recovery Test using Bio Surfactant of Halo Tolerant Bacteria Brevundimonas

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Oil Recovery Test using Bio Surfactant of Halo Tolerant Bacteria *Brevundimonas diminuta* and *Bhurkholderia glumae* at Variation of NaCl Salt Concentrations

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Oil recovery test has been done by using crude biosurfactant from *Brevundimonas diminuta* and *Bhurkholderia glumae* indigenous halo tolerant bacteria with the variation of NaCl salt concentration 0; 1.5; 3; 4.5; 6; and 7.5%. Oil recovery test was obtained by determining % TPH (Total Petroleum Hydrocarbon). The sample concentration was 28.19% TPH, it was extracted by using biosurfactant of *Brevundimonas diminuta* and *Bhurkholderia glumae* bacteria, the optimal salinity conditions were at 3, 4.5% salt concentrations with the value oil recovery as much as 50.41, 69.97 % respectively. Oil components which extraction by biosurfactant were analyzed by using GC-MS (*Gas Chromatography-Mass Spectrophotometry*). The result from analyzes GC-MS could be concluded that bacteria *Brevundimonas diminuta* could dissolve hydrocarbon compounds short chain carbon atom at fraction $<C_{10}$–C_{14} and long chain carbon atom at fraction $>C_{22.}$ $<C_{10}$, C_{11}-C_{14} and C_{15}-C_{17} and *Bhurkholderia glumae* could dissolve hydrocarbon compounds short chain carbon atom at fraction $<C_{10}$–C_{14} and long chain carbon atom at fraction $>C_{22}$ according to the retention time.

Key words: *Bhurkholderia glumae*, *Brevundimonas diminuta*, crude biosurfactant, indigenous halo tolerant bacteria, recovery oil

Uji coba rekoveri minyak telah dilakukan dengan menggunakan biosurfaktan kasar dari *Brevundimonas diminuta* dan *Bhurkholderia glumae* yang merupakan bakteri asli Indonesia halo toleran dengan konsentrasi konsentrasi garam NaCl 0; 1.5; 3; 4.5; 6; dan 7,5%. Uji perolehan minyak diperoleh dengan cara menentukan % TPH (*Total Petroleum Hydrocarbon*). Konsentrasi sampel adalah 28,19% TPH. Sampel diekstraksi dengan menggunakan biosurfaktan bakteri *Brevundimonas diminuta* dan *Bhurkholderia glumae*, kondisi salinitas yang optimal berada pada konsentrasi garam 3, 4,5% dengan nilai perolehan minyak masing-masing sebanyak 50,41, 69, dan 97%. Komponen minyak yang diekstraksi dengan biosurfaktan dianalisis dengan menggunakan GC-MS (*Gas Chromatography-Mass Spectrophotometry*). Hasil analisis GC-MS dapat disimpulkan bahwa bakteri *Brevundimonas diminuta* dapat melarutkan senyawa hidrokarbon rantai pendek atom karbon pada fraksi $<C_{10}$–C_{14} dan atom karbon rantai panjang pada fraksi $>C_{22.}$ $<C_{10}$, C_{11}-C_{14} dan $^{C}{}_{15}$-$^{C}{}_{17}$ dan *Bhurkholderia glumae* bisa melarutkan senyawa hidrokarbon atom karbon rantai pendek pada fraksi $<C_{10}$–C_{14} dan atom karbon rantai panjang pada fraksi $>C_{22}$ sesuai waktu retensi.

Kata kunci: bakteri asli Indonesia yang halo toleran, *Bhurkholderia glumae*, biosurfaktan ekstrak kasar, *Brevundimonas diminuta,* perolehan minyak

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World's petroleum demand will increase from year to year along with the rapid development of global economy. For that the oil industry players must continue to work to meet these demands. But the problem is that oil production is limited by the value of recovery factor (RF) that is a ratio showing the amount of oil that can be produced on the surface. This value will limit the amount of oil that can be produced by the primary recovery mechanism. The oil production is very dependent on the characteristics of reservoir and fluid and the type of driving mechanism that helps provide the thrust to the oil to flow to the surface (Lazar *et al*. 2007;Shibulal *et al.* 2014).

In order to increase cumulative oil production this acquisition should be increased as much as possible. The way is by applying the EOR (Enhanced Oil Recovery) method to the reservoir. The principle is to use external power to the reservoir so it is hoped that the power can help lifting the oil to flow to the surface. The EOR is chemical surfactant and one of the EOR technique developed now is by utilizing microbes known as Microbial Enhanced Oil Recovery (MEOR). The MEOR method is more favorable than the EOR

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method because this method can extract petroleum more efficient and environmentally friendly. The potential of microorganisms to degrade heavy crude oil to reduce viscosity is considered highly effective in MEOR (Lazar *et al.* 2007; Enas 2007). The basic principle of MEOR is the utilization of microbial secondary metabolite products to help increase the remaining oil gain or still trapped in the reservoir.

Bacterial decomposition by MEOR can include organic, inorganic acids, gases, water and bio surfactants. Bio surfactant is one of the products produced by microbes and plays an important role in oil recovery because it reduces surface tension between two-phase fluids, thereby increasing oil mobilization (Sea and Dhail 2013; Chandankere *et al.* 2013).

The ability of bacteria to produce bio surfactants is related to the ability of bacteria to use hydrocarbons as their substrate. Microorganisms with large bio surfactant production generally have a great ability also in decomposing hydrocarbons. Types of growth substrate, bacteria type, source of nutrition and environment are the main factor of the researcher's attention in optimizing bio surfactant production. Salt is an important environmental factor for bacterial growth and development. If the salt content of the environment is not compatible with microbial enzyme activity, bacteria can not be metabolized properly so it does not grow optimally (Sarafin *et al.* 2014; Shin *et al.* 2001; Garcia-Blanco *et al.* 2007).

Bhurkholderia glumae and *Brevundimonas diminuta* bacteria belong to potential bacteria as biosurfactant and degradation producer (Yudono *et al.* 2010; Yudono *et al.* 2011). The presence or absence of crude biosurfactants that have been produced from bacteria with a density of ± 10^7 cells mL^{-1} was tested using hemolysis tests (Dhail 2012). Potential bacteria in producing biosurfactant, based on the size of the inhibit zone / clear zone formed by test bacteria on blood agar. Known both bacteria have the ability to produce a good biosurfactant because it has a clear zone that is 25.96 mm. A clear zone formed over 20 mm indicates that the bacteria tested have good potential for producing biosurfactants (Yudono and Estuningsih 2013). Both of these bacteria are also bacteria whose growth depends on the concentration of NaCl so it is potentially also possible in producing optimal bioproducts at certain salt levels (Shin *et al.* 2001). Each bacterium has resistance to different salts while oil wells in Indonesia mostly have salinity characteristics (up to 40,000 ppm) (Almeida *et al.* 2004; Hao *et al.* 2008). For certain concentrations of NaCl also causes a decrease in water-surface tension by surfactants, this is because the chemical bonds that make up NaCl are ionic bonds capable of affecting hydrophobic and hydrophilic groups in lowering inter-phase surface tension (Mnif and Ghribi 2015; Rufino *et al.* 2014). Both bacteria have good tolerance to salinity concentration up to 7.5%.

This research will be conducted in variation of salt content of 0%; 1.5%; 3%; 4.5%; 6%; and 7.5% in biosurfactant for recovery in petroleum. The resulting biosurfactant was tested with a sludge sample, then the oil recovery was calculated using TPH calculation (Total Petroleum Hydrocarbon). To see the components of the degraded hydrocarbon compounds, a GC (Gas Chromatography) analysis of sludge produced the best% recovery (at the best salt level) of each bacterium (Ibrahim *et al.* 2013).

MATERIALS AND METHODS

Materials. The *Brevundimonas diminuta* and *Bhurkholderia glumae* bacteria were isolated from oil field Babat Toman Village, sludge oil (obtained from Babat Toman Village, Muara Banyuasin, South Sumatera), and molasses was taken from sugar waste of PT Cinta Manis, Tanjung Raja, Ogan Ilir, South Sumatra).

Maintenance of Culture. A total of 1 ose of each bacterium *Brevundimonas diminuta* and *Bhurkholderia glumae* were inoculated to solid medium NA with zig zag movement. The bacteria that have been inserted into the NA medium are incubated in the incubator for 24 h. After incubation bacteria ready to use.

Medium Preparation. Zobell media was prepared 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 aquadest with a volume of 1000 mL solution. The mixture is boiled over the hotplate and homogenized with a magnetic stirrer. After boiling the sterilized mixture with autoclave at 121 °C for 15 min (Sunitha *et al.* 2007; Behlugil 2002).

Bacterial Starter. *Brevundimonas diminuta* and *Bhurkholderia glumae* culture cultures were taken 5 ose, then subcultured into each erlenmeyer containing 100 mL of medium Zobell, then aerated for 24 h, after 24 h of aeration was stopped. Zobell's 100 mL was added to the mixture until the total volume is 200 mL and the mixture is re-aerated until the shortest generation time of *Brevundimonas bacteria* and *Bhurkholderia glumae* for 12 h.

Production of Crude Biosurfactants . The production process of crude biosurfactant is done by

mixing bacterial starter, Medium Zobell and 15% molasses concentration for *Brevundimonas diminuta* bacteria and 20% for *Bhurkholderia glumae* bacteria with a total volume composition of 200 mL. The mixture was incubated based on the shortest generation time of each bacteria ie 12 h.

Biosurfactant Preparation with Variation of Salt Level Concentrations. The process of making salt-containing biosurfactant solution is done by mixing crude biosurfactant from *Brevundimonas diminuta* bacteria and variation of NaCl 0%, 1.5%, 3%, 4,5%, 6%, 7,5%. The same treatment was performed using *Brevundimonas diminuta* and *Bhurkholderia glumae* bacteria.

Crude Biosurfactant Application on Sludge for Oil Recovery. A total of 25 g sludge (obtained from Babat Toman village, Musi Banyu Asin, South Sumatra) (b/v), were incorporated into each biosurfactant with variations of 0% NaCl salt, 1.5%, 3%, 4.5 %, 6% and 7.5% (w/v against biosurfactant) to a total volume of 200 mL. The mixture is then aerated for 10 d. The mixture is then filtered with filter paper. The residue from the filtration result is then calculated by the final percentage of TPH.

TPH measurement. 250 mL boiling water with a socket-shake extract to be used is dried in the oven, then cooled in the desiccator. A total of 10 g sludge was inserted using a filter paper of the appropriate size. The filter paper containing the sludge sample is then inserted into the socket tube, then the top of the socket tube is connected to the condenser and the bottom is connected to a boiling flask containing n-hexane solvent with the volume wetting the entire filter paper. The sludge sample is then extracted until the solvent descends back into the boiling flask until it is clear.

GC analysis. GC analysis was performed on sludge which yields the largest% oil recovery in each bacteria by analyzing GC sludge chromatogram before and after treatment at 0-7,5% salt. The type of column used is TG-5MS with a column length of 30 m and a diameter of 0.25 mm (Yudono 1994). The samples were taken by several µL, to be injected to a Thermo Scientific GC tool with a pre-program temperature of 40 °C maintained for 4 min, the temperature was raised 5 °C every 1 min until the temperature reached 300 °C.

Data analysis. The data to be obtained from this research are, among others, the initial sludge TPH measurement data to determine the percentage of hydrocarbon compounds present in sludge prior to treatment and final TPH data after the treatment of salinity variation and then compared with the initial TPH values to see the biosurfactant ability to dissolve the hydrocarbon compound on sludge.

The data obtained from the experiment was processed by using ANOVA (Analysis of Variance) analysis to test the difference of salt and oil recovery.

RESULTS

Total petroleum hydrocarbons (TPH) were measured to determine the percentage of hydrocarbon compounds present in petroleum. Measurements of Total Petroleum Hydrocarbons (TPH) in oil-contaminated sludge were used as samples and parameters to determine the bio surfactant's ability to reduce further TPH values. Initial TPH measurements on sludge samples were performed by solvent extraction using n-hexane and using sochletation method (Margesin, Hämmerle and Tscherko, 2007). The initial TPH data generated from the measurement of 28.19%. The samples were treated with the biosurfactants of both bacteria that had varied NaCl salt by 0; 1.5; 3; 4, 5; 6; 7.5%. The results are characterized by reduced oil sludge levels after treatment. In this study, the biodegradation process is known from the calculation of heavy TPH oil sludge from residual weight (oil residual) sludge weight, then compared with the initial weight added by gravimetric method, the results were presented in Table 1.

Result of Analysis of Petroleum Hydrocarbon Compound on Sludge using GC-MS. The following chromatograms show the initial components of sludge (before the treatment of variation of NaCl salt content), sludge chromatogram with control, chromatogram sludge which having optimal NaCl content yielding the largest petroleum recovery, and a histogram of oil abundance in each bacterium. ThermoScientific GC-MS analysis results are shown in Figure 1.

DISCUSSION

Based on Table 1, the final% TPH value after biosurfactant treatment with variation of salt concentration's of NaCl has different TPH value respectively. As result, the salt concentration can affect the work of biosurfakan in ddissolving sludge.

The optimal NaCl concentration was 3% for *Brahmanimonas diminuta* and 4.5% for *Bhurkholderia glumae* bacteria. The higher levels of NaCl salt that affect will result in different percentages of petroleum recovery, where salinity is one of the natural environmental factors present in the sludge sample area

Table 1 The average percentage of TPH extracted from 28.19% TPH Samples

Salinity NaCl (%)	%TPH(average) *B. diminuta*	*B. glumae*
0	8.3283	6.7539
1.5	10.8486	9.8157
3	14.1992	7.2273
4.5	7.7921	19.7209
6	9.3863	14.2114
7.5	8.3641	7.7767

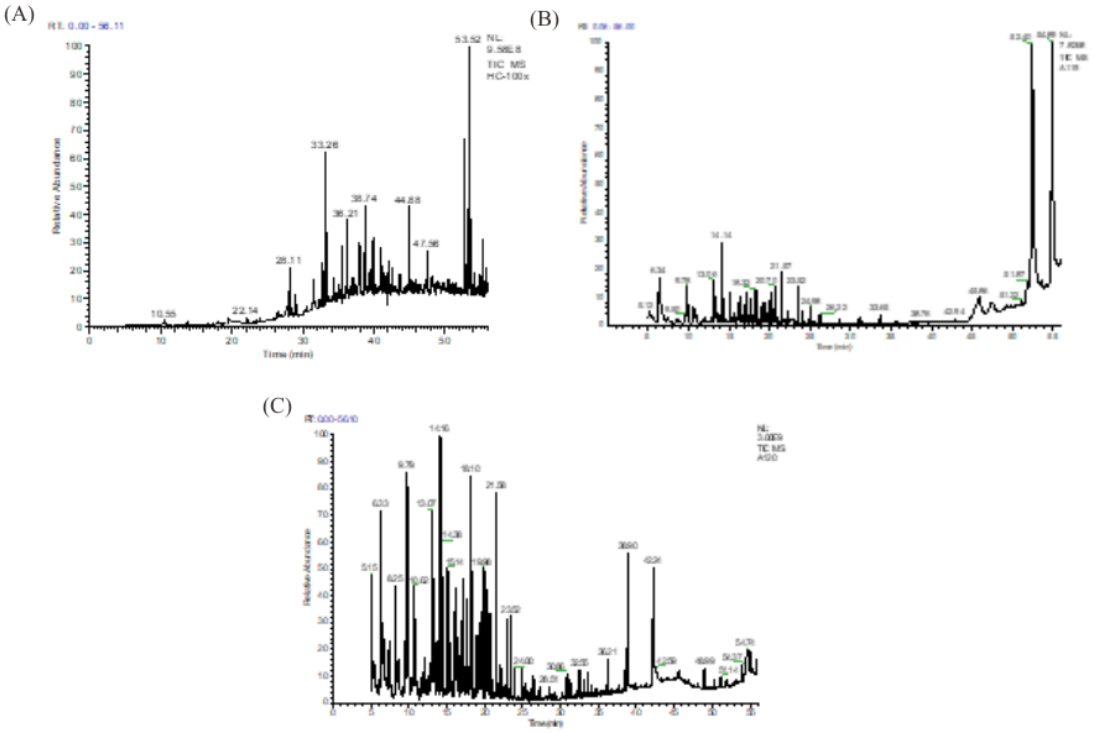

Fig 1 Early TPH chromatogram prior to the treatment of crude biosurfactant (A), final TPH chromatogram after treatment using crude bosurfactant with variation of 3% salt on *Brevundimonas diminuta* bacteria (B), final TPH chromatogram after treatment using crude bosurfactant with variation in salt content 4.5% on the *Bhurkholderia glumae* bacteria (C).

(Shin *et al.* 2001; Garcia-Blanco *et al.* 2007).

From the data in Table1 and described in Figure 2 show that the two bacteria produced different biosurfactant yields at each salt concentration, in which the *Bhurkholderia glumae* bacteria produced a more optimal biosurfactant compared with *Brevundimonas diminuta* bacteria. The Fig 2 shows following;

This indicates that the bacterium *Bhurkholderia glumae* is an effective bacteria for oil recovery because the resulting biosurfactant can increase the solubility of hydrophobic compounds so as to increase and accelerate the rate of degradation by microbes. Appropriate salt content will lead to a faster biodegradation process from bacterial culture. Similarly, the type of bacteria itself will affect changes in salt levels obtained from bacterial metabolism activities. And vice versa if the level of salt is not suitable then the activity of bacteria will decrease that will affect the total of bacterial cells (Shin *et al.* 2001). It is also revealed by Charlena (2010) that salt conditions affect the growth of bacteria in degrading petroleum hydrocarbons. Optimum salt conditions will enhance the species' ability to degrade hydrocarbons.

Data analysis were done by using data analysis

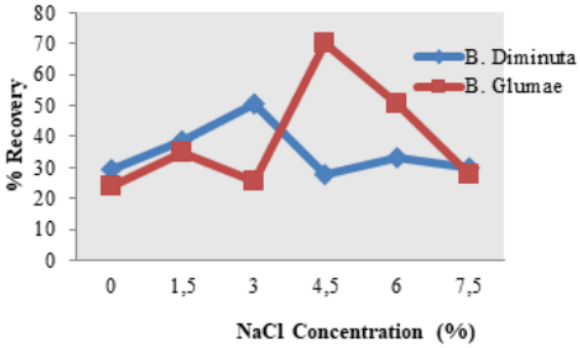

Fig 2 Graph of petroleum recovery results after addition of salt content of NaCl in *Brevundimonas diminuta* and *Bhurkholderia glumae bacteria.*

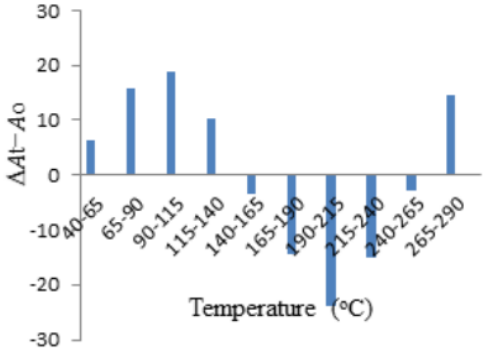

Fig 3 Histogram changes of dissolved hydrocarbons before and after addition of crude biosurfactants from *Brevundimonas diminuta* bacteria.

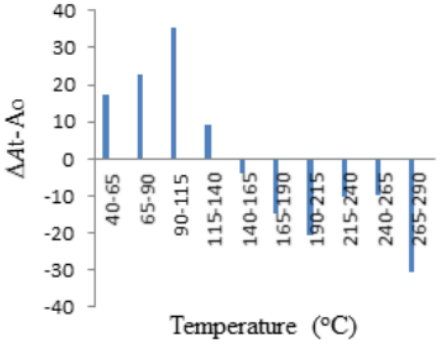

Fig 4 Dissolved hydrocarbon histogram before and after addition of crude biosurfactant from *Bhurkholderia glumae* bacteria.

Table 2 Chain fraction C based on its temperature

Temperature Range	The identified chain C fraction
<100 C	$<C_{10}$
100-150 C	C_{11}-C_{14}
150-200 C	C_{15}-C_{17}
200-250 C	C_{18}-C_{21}
250-300 C	$>C_{22}$

(Yudono 2014)

with anova method, it shows that F value count on *Brevundimonas diminuta* and *Bhurkholderia glumae* bacteria bigger than F critical value, where F arithmetic for *Brevundimonas diminuta* bacteria has value of F count 59, 24098 while bacteria Bhurkholderia glumae has a value of F arithmetic 87, 5864. The value of Least Significant Different test value is higher for each bacteria, where it can be concluded that any difference in salt content of NaCl gives a very significant oil recovery.

Result of Petroleum Recovery test by Using control. Petroleum Recovery Test using aquades is used as a control to be applied to a sludge sample that aims to determine the biosurfactant's ability to dissolve petroleum. The result of oil recovery at control that is equal to 4,7321%. The results of the recovery using the aquades will then be analyzed using GC to see components of the hydrocarbon compound after treatment, and used as control.

GC-MS analysis is only performed for the treatment of the largest petroleum recovery results using biosurfactant with optimal saline NaCl content obtained from each bacterium. In addition, GC analysis was conducted on sludge with treatment using aquades that served as a control to see the working ratio between biosurfactant and aquadest. Objective GC-MS analysis can provide information on the amount of the constituent components of petroleum before extraction and after the extraction using crude biosurfactant with variation of salt content (Ibrahim *et al.* 2013; Jovančićević *et al.* 2004).

In Fig 1a Early TPH chromatogram prior to the treatment of crude biosurfactant in obtaining the petroleum constituent component before treatment showed the presence of 16 peaks of the petroleum compound based on a total retention time of 56 min, whereas in Fig 1b final TPH chromatogram after treatment using crude bosurfactant with a variation of 4.5% salt on *Brevundimonas diminuta* bacteria showed the presence of 15 petroleum compound peaks based on a total retention time of 56 min. Fig 1c final TPH chromatogram after treatment using crude bosurfactant with a 3% salt content variation in the *Bhurkholderia glumae* bacteria showed 21 peaks of the petroleum compound based on a total retention time of 56 min. It can be concluded if viewed from the peak amount after the treatment compared before the treatment of the addition of the peak amount that appears on the chromatogram. To see the degradability of hydrocarbon compounds can be seen from the change in the concentration of the initial hydrocarbon compound before treatment and the end after treatment. Concentration changes can be analyzed using gas chromatography in the form of peak areas. Of these differences may indicate a change in peak areas at baseline and after addition of biosurfactant (Penet *et al*. 2006).

If the chromatogram (a) is compared with (b) it can be seen that there is a decrease in the peak area detected at retention time 25-50 min. Then at 5-25 min retention periods small peaks indicate the presence of newly undetectable compounds. The increase is considered to be the result of degradation of a high molecular weight compound which then dissolves into a low molecular weight so that it appears at an earlier mooring time, resulting in a decrease in viscosity. This decrease in viscosity causes oil mobility to increase (Li *et al*. 2002) (Hao *et al.* 2008). While in figure (b) is a chromatogram of petroleum component of *Brevundimonas diminuta* bacteria with the best salt concentration of 4.5% NaCl compared with chromatogram of petroleum constituent components before treatment (a) there is a decrease of peak area areas at retention time 25-55 min, the decline in area is suspected because the bacteria can directly use and degrade the hydrocarbon compound into a simple compound. While at 5-25 min retention time new peaks appear degradation of C length to C short with the addition of wide peak area (Penet *et al*. 2004).

From the Figure 1 indicates that beginning and after adding biosurfactant further decreases the peak area of the chromatogram. The peak areas decrease in chromatogram are caused by the breakdown of hydrocarbons into simple compounds. This is largely determined by the type of bacteria, where each bacteria has different capabilities in degrading the petroleum sludge. From the GC-MS data obtained, soluble and insoluble hydrocarbon analyzes that are left on the residue are shown on Figure 3 and Figure 4 the histograms of percent abundance of each bacteria.

The soluble component in the biosurfactant and the residual component can be explained through the histogram of the abundance change. Based on the histogram, the x axis is the temperature calculated from the retention time and the y-axis is the change of abundance. The histogram was derived from obtaining initial chromatogram data of the compound and after treatment of variation of salt NaCl content in each bacteria, by comparing initial abundance before treatment and after variation of salt content of NaCl based on retention time. Changes in abundance are indicated by ΔA =% At-% Ao if the resulting data is a

positive number so it indicates the hydrocarbon chain component dissolved in biosurfactant, and if it is a negative number it indicates that the hydrocarbon chain component becomes residue. The carbon chain fraction contained in the chromatogram can be identified based on the program temperature methodology (Yudono *et al.* 2010). The carbon chain fractions can be grouped according to the temperature range of the program as shown in Table 2.

The component changes before and after the treatment are described qualitatively in the abundance change shown in Figure 3 in the form of a histogram image.

Based on the Figure 3 calculated the percent difference of abundance by subtracting percent abundance after addition of crude biosurfactant (At) from *Brevundimonas diminuta* bacteria minus percent of peak abundance before addition of crude biosurfactant (A_0). A positive reduction results indicate that the hydrocarbon compound dissolves in the biosurfactant. An increased histogram shows that short-chain hydrocarbon compounds dissolve in biosurfactant, so that at the top of the chromatogram there is a missing carbon chain and breaks down into short chains. This histogram explains that the biosurfactant of the *Brevundimonas diminuta* bacteria based on its retention time is capable of dissolving short C chain hydrocarbons ie the $<C_{10}$-C_{14} atomic chain at 40-140 °C and dissolving the long C chain hydrocarbons ie the atomic chain $>C_{22}$ at a temperature of 265-290 °C but the biosurfactants of the *Brevundimonas diminuta* bacteria are not able to dissolve the long C chain hydrocarbons C_{15}-C_{17} and C_{18}-C_{21} at the temperature of 140-265 °C.

The histogram shows analyzing the soluble and insoluble-degredated components qualitatively as in Figure 4.

This histogram shows that the treatment with biosurfactant from Bhurkholderia glumae bacteria based on retention time is not capable of dissolving long chain C hydrocarbon compounds ie C_{15}-C_{17}, C_{18}-C_{21}, and $> C_{22}$ at a temperature of 140-290°C so that the component remains as residue. Furthermore, biosurfactant can dissolve the hydrocarbon chain compound of short C atom, ie, the atomic chain $<C_{10}$, C_{11}-C_{14} and C_{15}-C_{17} at temperature 40-140°C.

The chromatogram ratio of petroleum and recovery chromatograms with biosurfactants from each bacterium has a peak change. Changes in the peaks of chromatograms can occur due to the degredation process by bacteria. The degredation results may indicate a missing, emerging peak, larger peak height or peak height reduction. Missing peaks show that the hydrocarbon chains demineralized into CO_2 dan H_2O (Bharali *et al.* 2011).

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