Rhamnolipid NR22 Green Detergent by *Pseudomonas aeruginosa* NR22

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***Abstract*— Washing detergent consisted of variety of components as example surfactants and enzyme. Green Alternative or biodegradable detergent has been introduced by using *Pseudomonas aeruginosa* to produce biosurfactant (green detergent). This research started with the preparation of rhamnolipid biodetergent and the chemical structures of the surfactants product was confirmed by Fourier Transform Infrared Spectrometer (FTIR). The results show only 40% of foaming formation can be obtained and the foam could remain to 5 hours which qualified as the good stability product of biosurfactant. Absorption valleys at 3286.26 and 3308.81 cm^{-1} in commercial and produced biosurfactant respectively shows the O-H band bond as hydroxyl groups. Another broad absorption valley observed in the value of 2151.11 and 2159.97 cm^{-1} were demonstrated in stretching C-C bond which was in alkyl groups. Absorption at 1637.85 cm^{-1} for the commercial biosurfactant and 1634.89 cm^{-1} for the produced biosurfactant was characterized as stretching C=C bond of alkene groups. A bending of C=O bond at 1737.26 and 1365.16 cm^{-1} for produced biosurfactant were in a group of carboxylic acid. The wavelength of 1364.82 cm^{-1} for the commercial biosurfactant also came from the same group of carboxylic acid. The absorption at 1217.02 cm^{-1} for the produced biosurfactant consisted the stretching of C-O bond which in hydroxyl group. The percentage oil removal of 94.87% was excellent using the formulation of biodetergent which was nearly similar to the commercial detergent (99.5%) and mix of commercial detergent with biodetergent (96.6%). The trend of the graph of oil removal percentage versus temperature of washing medium shows as increasing in temperature of washing medium, the percentage of oil removal on the cotton cloth also increasing. Rhamnolipid NR22 have the capability and high potential as a good biosurfactant and detergent in comparing the images using Scanning Electron Microscopy (SEM).**

Keywords*— Biosurfactant, Rhamnolipid, Detergent, *Pseudomonas aeruginosa

I. INTRODUCTION

Washing detergent consist of variety of components as example surfactants and enzyme. The major functions of surfactant are reducing surface tension between two different phases and interrupt the bonding, solubilize the dirty marks and lastly for preventing redeposit. Surfactants have several number of application besides apply it for daily use, surfactants also can be used in agriculture, health and in most industry [1-2]. Other than surfactants, the contents inside of any type of detergent mostly are fillers, builders and some of the detergent consists of variation of supplementary materials based on the product itself and usability. The surfactants that found in washing detergent plays an important roles in removing dust, mark or smudge from cloth or textile by decreasing the interfacial tension [3].

Detergent is a surfactant that consists of cleaning properties and one of the cleaning agent that water soluble. Detergent is a cleaning agent that helps to remove the detachment of stains, dirt, blood or grease on cotton fabric or clothes or on the surface of metals, woods or plastics. Laundry detergents is example of detergent that contains cleaning agent for cleaning laundering. Detergent comes in powder and liquid form while some of the company produce liquid detergent pods that is more effective and convenient since it is in a small packaging. The major components of every detergents are builders, surfactants and fillers. Detergents has been used most industrial that make them as important cleaning product with an investment of $60 billion per year in globally [4]. The composition of usually consists of surfactants, builders, fillers, bleach and enzymes [5]. Some of detergent's company did an addition of additives into their ingredient of detergent to enhance their product and more commercialized among the competitors. The function of the additives includes modified foaming effectiveness, soften the cloth, sweet fragrance and improve the optical brighteners. Surfactants and enzymes are major components for cleaning actives [6].

Surfactants is one of the major components in detergent that responsible for washing performance. Generally, surfactants is a components which lower down the surface tension between two phases of components such as between two liquids (oil and water) [7]. Therefore, surfactants play an important roles in removing any dirt or grease on the surface on cotton fabric. Surfactants can be a good components in laundry detergent formulations as it proves that oil can be separate with water in an oil solution [5].

The usage of enzyme inside detergent has been discovered by Otto Rohm back in 1913. Generally, the dirt and stain on our clothes consists of two types of dirt which are organic and inorganic. Dust is one of the example of inorganic dirt that can separate easily from the surface of cotton cloth without any enzyme in the ingredient of detergent. It comes to a problem when handling the organic dirt because of its properties of insoluble in water and hard to remove the stain or dirt on the surface of the cloth [8]. As for that, commercial detergent nowadays has been upgrading the detergent formulation as well as it increasing the washing performance and effectiveness. Besides its ability to remove the difficult dirt, it also one the way to minimize the cost of energy [7]. Enzyme is able to minimize or substitute the surfactants itself within detergent [8]. Protease and lipase are the example of enzyme that commonly use in detergent.

The alteration of detergent formulations have given rise to the negative impacts to the environmental predominantly in wastewater from the household or industrial waste [9]. Currently, the detergents that been used comes from non-renewable petrochemical resources and lead to other issues such as toxicity, low biodegradability, allergenicity and bad pollution. The term green detergent or biosurfactant is the most suitable name for the products as it is has been verified to have a high biodegradability, decreases the toxicity and high selectivity to have a better environmental impact [10].

Biosurfactant can be organized into chemical composition of

biochemical nature and types of microbial producer. Each compound contains different polarity and structure. There are many types of biosurfactant that can be produced from variety of microorganisms as example glycolipids, lipopeptides, fatty acids, surfactin, phospholipids and others. Different types of structure will assists to dissimilar of characteristics of each compounds.

Glycolipids

The most compound that produced biosurfactant is glycolipids. Glycolipids is made up from microorganisms called *Pseudomonas aeruginosa* and *Pseudomonas sp.* which usually to produce rhamnolipids. It consists of a long chain of hydroxyl aliphatic acids and also carbohydrates. Besides microbial sources, rhamnolipids also can also be generated by some of carbon origin for example glucose, ethanol and vegetable oil [11]. Besides rhamnolipids, glycolipids also known as sophorolipids and trehalolipids. Sophorolipids and trehalolipids can be produced by Candida apicola and Arthrobacter paraffineus respectively.

Lipopeptides

Generally, lipopeptide is a compound that contains a connection between lipid and peptide. The combination of microorganisms *Bacillus* and *Pseudomonas* could build the strains to produce lipopeptides biosurfactants. Besides bacterial strains, crude oil from the carbon origin may also bring out the product of lipopeptide biosurfactant during fermentation [12]. Lipopeptides are group of surfactin that widely known for the most possible in making biosurfactant from Bacillus subtilis. Surfactin consists of concentrations beneath 0.005% reduce surface tension to 27 mN/m that proves surfactin is redoubtable among other type of biosurfactants [13]. A study has been proved that in isolation from oil reservoir, a genus from Bacillus have the ability to produce biosurfactants [14].

Fatty Acids, Phospholipids and Neutral Lipids

During growth of n-alkanes, assorted number of yeast and bacteria can build a huge quantities of fatty acids and phospholipids. The hydrocarbon chain's length always interconnected with the hydrophobic and hydrophilic structure for their own structure. The chemical compositions and the structure of this biosurfactants are rely on some factors such as the microorganisms produced, the nutrients from the culture medium and its growth surroundings [15].

Polymeric Biosurfactants

Polymeric biosurfactants are made up from fatty acids that connected with repeating sugars. It has high molecular weight and most commonly polymeric biosurfactants are lipomanan, emulsan, alasan and liposan. Emulsan is very productive in emulsification in hydrocarbons. It is capable in decreasing the surface and interfacial tension of water as well as 100% of removal percentage in tube cleaning at ambient temperature [16].

Pseudomonas aeruginosa is one of microorganisms that can be obtain and isolate from any sources for example soil, plants and water. Unfortunately, this type of microorganism may cause infections to human that act as pathogen. It is only applicable when the human's skin was disrupted with wounds and burns [17]. *Pseudomonas aeruginosa* is one of the microbial source that produce rhamnolipids as biosurfactants. *Pseudomonas aeruginosa* has the ability to produced rhamnolipids. The biosynthesis of rhamnolipids was depending on the nutritional conditions and environmental of the microbial growth [18].

Green alternative has been introduced by using *Pseudomonas aeruginosa* NR22 (Ps.NR.22) to produce biosurfactant rhamnolipid (green detergent). This type of green biosurfactant (Rhamnolipid NR22) is high biodegradability, withstand at extreme pH and temperature and also minimize the toxicity. This study was aimed to determining the effectiveness of green detergent or cleaning efficiency of biodetergent from *Pseudomonas aeruginosa* compare to other commercial detergents.

II. METHODOLOGY

A. Materials

All material and chemicals were collected from various sources and quality as listed. *Pseudomonas aeruginosa* NR22 (bacteria), *Pseudomonas* powder (Microbiology), nutrient broth (Merck), potassium sulphate (K_2SO_4, Parchem), glucose ($C_6H_{12}O_6$, Sigma-Aldrich), bacteriological peptone (Ultrapure, protein = N×6.38 ≥ 76.5%), ammonium dihydrogen phosphate (CAS Number 7722-76-1, ≥98%, $NH_4H_2PO_4$, Sigma-Aldrich), magnesium sulphate heptahydrate (MgSO4.7H2O, ≥98%, Sigma), ammonium sulphate ($(NH_4)_2SO_4$, 99.5%, Molbase), potassium chloride (KCl, 99%, Vetec) and tris-hydrochloric acid (≥99%, Sigma-Aldrich).

B. Preparation of Rhamnolipid NR22 Green Detergent (Biosurfactant) from Ps.NR.22

To prepare the *Pseudomonas* agar, 32.5 g of *Pseudomonas* powder was mixed with 100 ml of distilled water and were boiled. It was followed by autoclaving for 2 hours. The mixture was poured into petri dish and keep into fridge for a night. Kay's minimal medium was prepared that contains 0.2 g of potassium sulphate, 0.1 g of magnesium sulphate heptahydrate and 0.2 g of glucose [19] with 100 ml of distilled water in a 200 ml conical flask. The pH of the mixture was adjusted to pH 6 by added sodium hydroxide. The Kay's medium was autoclaved in2 hours and stored into the fridge. 51 g of tris-HCl, 4.5 g of potassium chloride, 1.2g of magnesium sulphate3 g of bacteriological peptone and 15 g of glucose was mixed with 600 ml of distilled water was prepared for the peptone glucose ammonium salt (PPGAS) medium. The pH of the mixture was adjusted to pH 6 by added sodium hydroxide.

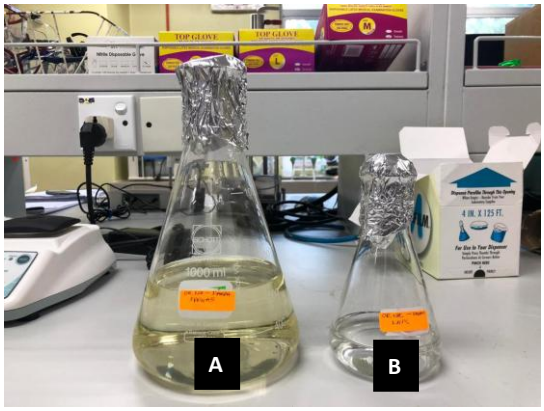

Fig. 1: (A) Proteose peptone glucose ammonium salt (PPGAS) medium and (B) Kay's minimal medium

The PPGAS medium was autoclaved for 2 hours and stored in the fridge. *Pseudomonas aeruginosa* NR22 bacteria was swapped onto the surface of agar as shown in Figure 2 and incubated at 37˚C with 100 rpm for 24 hours. The agar that turned to fluorescent green that contained *Pseudomonas aeruginosa* NR22 needed to be swapped and mixed together with nutrient broth in a test tube.

1 ml of nutrient broth was transferred into Kay's minimal medium and incubated for 48 hours at 37 ˚C with 300 rpm. 36 ml of Kay's minimal was then transferred into 600 ml of PPGAS medium. The PPGAS medium was incubated for 3 days in 37 ˚C with 300 rpm and production of biosurfactant (green detergent)

was observed. The percentage of foaming that formed after it has been shaked was calculated using the Equation 1:

Fig. 2: Bacteria swapped onto the surface of agar

$$Foaming\ percentage = \frac{Foam\ volume}{Total\ volume}\ x\ 100\%$$

[Equation 1]

The extraction of biosurfactant rhamnolipid NR22 was done by using centrifuge method. To remove the sample of *Pseudomonas* NR22 from the medium, the sample was placed inside conical centrifuge tube and centrifuge it for 15 minutes at 7,000 rpm in temperature of 15˚C.

C. Rhamnolipid NR22 analysis techniques

The sample of biosurfactant was confirmed by Fourier Transform Infrared Spectrometer (Model spectra 100 series, Perkin-Elmer Corporation, Norwalk, CT, USA). The characteristic of rhamnolipid NR22 was identified based on the structures and their chemical component. This classic method for structure analysis used IR light to induce an oscillation of chemical bonds at characteristic frequencies and energy was absorbed. IR spectra were recorded on a FTIR spectrometer in the 400–4000 cm^{-1} spectral region. Rhamnolipid that has been analyzed by IR spectroscopy often used technique of FTIR attenuated total reflectance (ATR) spectroscopy. To make a comparison, commercial biosurfactant was used in analyzing and comparing with the biosurfactant that being prepared for this study.

D. Washing Effectiveness of Green Detergent

In order to determine the washing effectiveness of the green detergent, 2 washing methods which are washing method between rhamnolipid NR22 green detergent and commercial detergent and washing method using rhamnolipid NR22 green detergent in different numbers of temperature. Both methods was adapted and modified using method by Khaje Bafghi [3]. Equation 2 is the equation to calculate the percentage of oil removal for each samples:

$$\text{Oil removal (\%)} = \frac{\text{Weight of oil absorbed into cotton cloth (g) [OVERNIGHT – BEFORE]} - \text{Weight of dry cotton cloth (g) [AFTER – BEFORE]}}{\text{Weight of oil absorbed into cotton cloth (g) [OVERNIGHT – BEFORE]}} \text{x100\%}$$

[Equation 2]

1) Washing Method between Rhamnolipid NR22 Green Detergent and Commercial Detergent

3 pieces of 2 x 2 cm^2 was cut. Each piece was stained with cooking oil. The pieces was kept at 25 ˚C for overnight. Before washing, each pieces was weighted. A beaker contained 2.0 g of biodetergent in 100 ml of tap water with a condition of pH: 7, temperature: 25 ˚C was shaked at 500 rpm for 20 minutes. The pieces require to rinse in 100 ml of purified water (distilled water) twice and dry the pieces at 20-25 ˚C. The removal percentage of each pieces was calculated by using the exact weight value before and after washing method by using Equation 2. A commercial detergent and mixture of green detergent were used with commercial detergent for this method to make comparison.

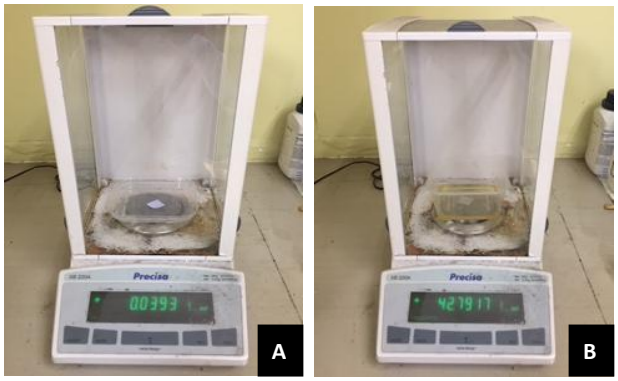

Fig. 3 Weighing the dry cotton cloth (A) and cotton cloth immersed in cooking oil (B) by using weight meter

2) Washing Method Using Rhamnolipid NR22 Green Detergent in Various Temperatures

3 pieces of 2 x 2 cm^2 was cut. Each piece was stained with cooking oil. The pieces was kept at 25 ˚C for overnight. Before the washing begun, each pieces was weighted. A beaker contained 2.0 g of biodetergent in 100 ml of tap water with a condition of pH: 7, temperature: 25 ˚C was shaked at 500 rpm for 20 minutes. The pieces required to rinse in 100 ml of purified water (distilled water) twice and the pieces dried at ambient temperature. The removal percentage of each pieces was calculated by using the exact weight value before and after washing method by using Equation 2. The stain pieces of cloth were washed in temperatures of 30, 40, 50 and 60 ˚˚˚C using the same steps of washing method.

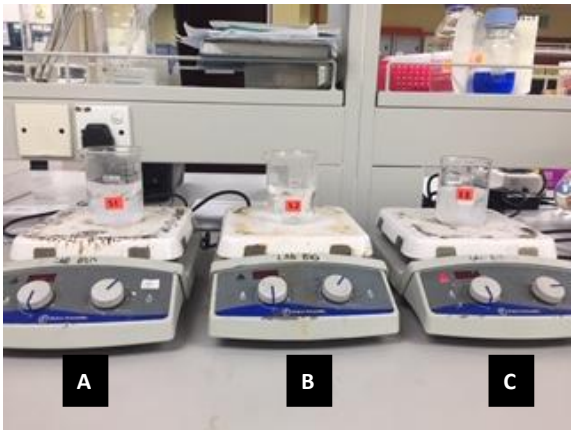

Fig. 4: 3 types of samples; commercial detergent (A), biodetergent (B), commercial detergent with biodetergent (C) undergoes washing process in a lab scale on hotplates

E. Effect of Washing Performance

The effect of washing performance on the washing method towards the surface of the pieces of cotton cloth can be determine by using Scanning Electron Microscopy (SEM) on JSM-5910 with 10 kV of acceleration voltage [20]. SEM was used to examine the images of the surface of cotton cloth after washed with biodetergent and commercial detergent.

III. RESULTS AND DISCUSSION

A. Production of Rhamnolipid NR22 Green Detergent

Figure 5 below shows the result of *Pseudomonas* agar that has turned to fluorescent green in colour after incubated for 24 hours and this proved that *Pseudomonas* aeruginosa microorganisms has been attached and grew on the surface of the agar plate. The green colour proved that the bacterial colony indicated a positive result of bacteria growth [21]. There are one or more pigments that can be formed by *Pseudomonas aeruginosa* strains which are pyoverdine (yellow-green and fluorescent), pyocyanin (blue-green) and pyorubin (red-brown) [22]. In order to make sure the biosurfactant complies and contain the characteristics of rhamnolipids, there are several types of conventional method in recovery the biosurfactant such as centrifugation, filtration, solvent extraction and acid precipitation [23].

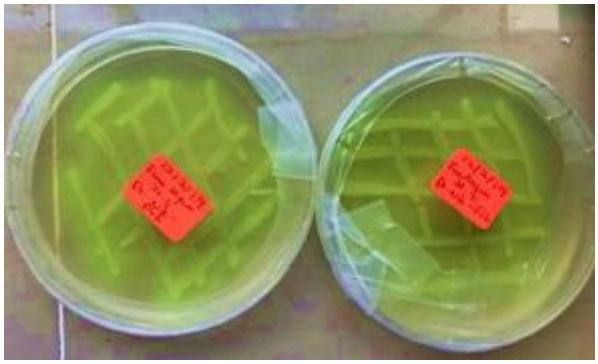

Fig. 5: The bacteria of *Pseudomonas aeruginosa* NR22 were grow in a stable condition

The nutrient broth shows in Figure 6 showed changes and differences before and after the incubation. The nutrient broth displayed an apparent result just from their appearance outside of both test tubes which the clear nutrient broth (A) switched to cloudy liquid (B). This demonstrated the growth of microorganism *Pseudomonas aeruginosa* inside the nutrient broth. The nutrient broth was represented as the food for the microorganisms and ingested all the nutrients inside the nutrient broth to create an enzyme throughout their process of consuming the nutrient broth. Several elements that effect the growth of microorganisms which are temperature, humidity and pH value of the food [24].

Foamability is one of the important basis or standard in preference of biosurfactant as biodetergent or emulsifier [25]. The foaming of rhamnolipids that have been produced is resulted from the vigorously stirring manually by hand shaking. As the result, Figure 7 shows the formation of foam after 2 minutes of shaking with uneven momentum. By using Equation 1, the foaming percentage was calculated based on the volume stated at Figure 7.

$$Foaming\ percentage = \frac{(640 - 385)\ mL}{640\ mL}\ x\ 100\% = 40\%$$

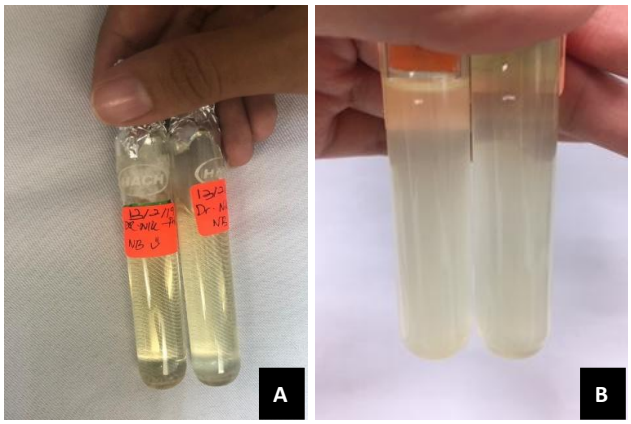

Fig. 6: The changes of nutrient broth from clear (A) to cloudy (B) from the appearance

The results show only 40% of foaming formation can be obtained from the mixture. Furthermore, the foam could remain to 5 hours which qualified as the good stability product of biosurfactant. The foam that produced exhibit the surface tension of an aqueous solution and due to the existence of biosurfactant, the air is decreased, thus the bubbles formed when two different phases mixed [26]. Foaming was very suitable to be the preliminary screening test for this research in identifying the production of biosurfactant by using microorganisms. The product of biosurfactant that comes from *Pseudomonas* microorganism can reached to 70% of foaming formation formation [27].

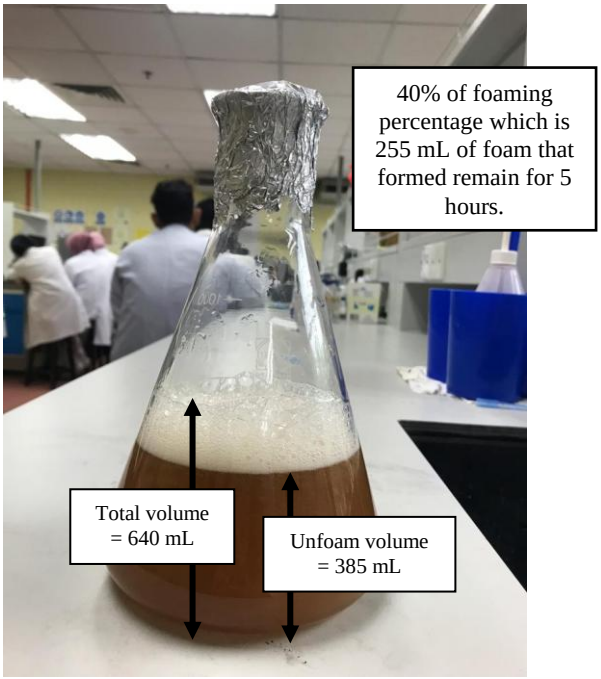

Fig. 7: Foams formed shows that rhamnolipid NR22 green detergent exits

B. Characteristic of Rhamnolipid NR22

Rhamnolipid NR22 consists of variety of functional group and group types in order to make the specific chain for rhamnolipid. This identification can be undertake by using Fourier Transform Infrared Spectrometer (FTIR) testing. Comparison of functional group has been made between the biosurfactant that being produced with the commercial biosurfactant. FTIR was a way of simply and rapidly quantify the biosurfactant whether it is in broth and mixtures. The peak area is the solution on the quantification of biosurfactant due to their carbonyl bond with internal standard in a concentration range. There are 6 types of peak area that can

observed and discussed in this characterization of rhamnolipid NR22.

Further with FTIR Spectrometry, Figure 7 shows the result of both samples for biosurfactants and red blocks show the difference between those 2 samples which there was a presence of a broad absorption valley at 1737.26 cm^{-1} in Figure 7B. This value of wavelength shows a bond of stretching C=O in the group of ester. The difference between both Figures were the presence of ester group in Figure 7B and this was due to the production of high concentration of fatty from Rhamnolipid NR22 by Ps.NR.22 [28]. Absorption valleys at 3286.26 and 3308.81 cm^{-1} in commercial and produced biosurfactant respectively shows the O-H band bond as hydroxyl groups that contained inside the biosurfactant. Another broad absorption valley observed in the value of 2151.11 and 2159.97 cm^{-1} were demonstrated in stretching C-C bond which was in alkyl groups.

Absorption at 1637.85 cm^{-1} for the commercial biosurfactant and 1634.89 cm^{-1} for the produced biosurfactant was characterized as stretching C=C bond of alkene groups. A bending of C=O bond at 1737.26 and 1365.16 cm^{-1} for produced biosurfactant were in a group of carboxylic acid. The wavelength of 1364.82 cm^{-1} for the commercial biosurfactant also came from the same group of carboxylic acid. While the absorption at 1217.02 cm^{-1} for the produced biosurfactant consisted the stretching of C-O bond which in hydroxyl group.

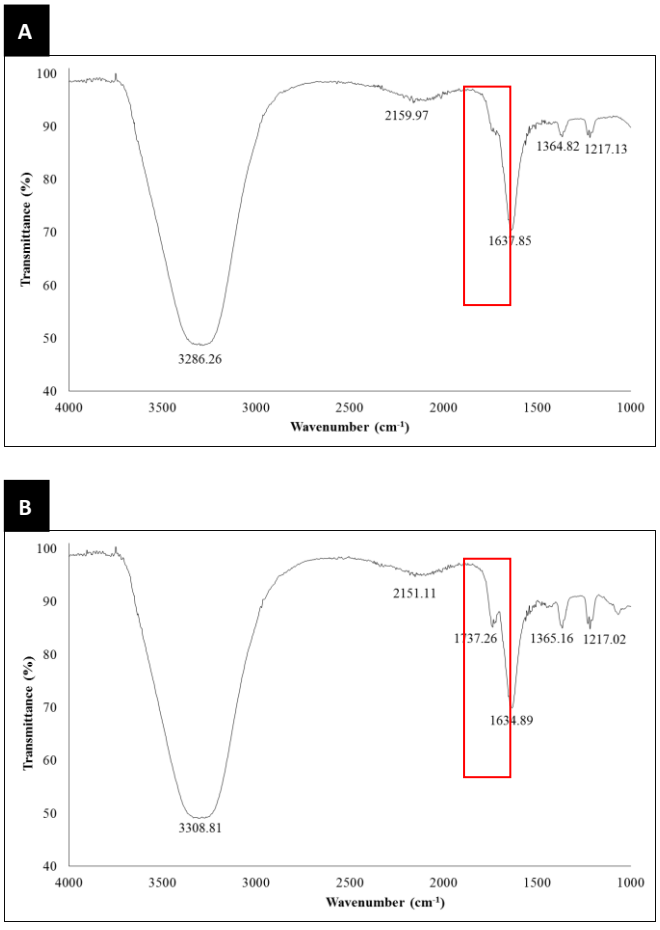

Fig. 8: FTIR spectra of biosurfactant sample: **A.** Commercial biosurfactant; **B.** Pseudomonas-produced rhamnolipids biosurfactant

C. Comparison with commercial detergent

3 types of samples have been prepared in making comparison between commercial detergent, biodetergent and combination of commercial detergent with biodetergent. Table 1 below shows the percentage of oil removal on a cotton cloth based on the precise weights before and after washing.

$$Average\ oil\ removal\ (\%) = \frac{\sum Oil\ removal\ (\%)\ for\ all\ trials}{3}$$

[Equation 3]

Equation 3 was used to calculate the percentage of average oil removal within the 3 time trials. This experiment work was repeated for 3 times to achieve the exact value since the value of those 3 samples were almost closed to each other.

Table 1: The percentage of oil removal on cotton cloth

Sample	Oil removal (%)			Average oil removal (%)
	1st trial	2nd trial	3rd trial	
Commercial detergent	99.6	98.9	100	99.50
Biodetergent (Rhamnolipid NR22)	94.9	94.9	94.8	94.87
Commercial detergent + biodetergent (Rhamnolipid NR22)	93.9	95.9	100	96.60

For this test, the formulation in this study was compared the Rhamnolipid NR22 with commercial detergent available on the Malaysian market. Figure 9 proved the results that the formulation of biodetergent that having the percentage of oil removal of 94.87% which was less effective as the commercial detergent that is 99.5% and mix of commercial detergent with biodetergent with percentage of 96.6%. However, the difference between the percentages was not very high. This is might due to the ingredients inside the biodetergent and commercial detergent. The formulation and ingredients of commercial detergent consists of variety of ingredients including enzymes. To enhanced the oil removal, lipases in commercial detergents [3]. Thus, the commercial detergents having a good performance of washing efficiency compare to the formulation of biosurfactant.

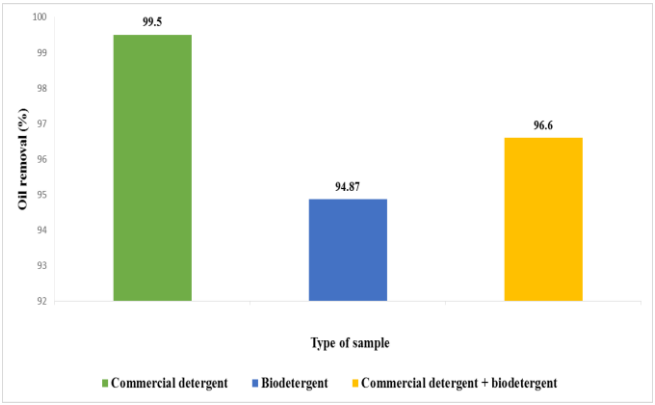

Fig. 9: Comparison of the formulation presented 3 types of samples for the removal of oil. pH = 7; temperature = 25 ˚C; stirrer velocity = 500 rpm; time = 20 min

D. The Effect of Temperature on the Effectiveness of Rhamnolipid

The same 3 types of samples were washed in different temperatures; 30, 40, 50 and 60˚C which are cold, warm, hot and extra hot in condition of washing respectively. Table 2 shows the percentage of oil removal according to the different temperatures on 3 types of samples.

Table 2: The percentage of oil removal on variety of temperatures

Sample	Temperatures			
	30 ˚C	40 ˚C	50 ˚C	60 ˚C
	Percentage (%)			
Commercial detergent	97	98.7	98.9	100
Biodetergent (Rhamnolipid NR22)	95	94.3	95.2	96.8
Commercial detergent + biodetergent (Rhamnolipid NR22)	96	96.8	98.5	98.7

Figure 10 shows the graph of oil removal percentage from cotton cloth in the function of temperature. The trend of the graph appeared as increasing in temperature of washing medium, the percentage of oil removal on the cotton cloth also increasing. High temperature may caused better in separation of oil in water. The commercial detergent has different types of components and have been formulated to increase the efficiency of washing performance. The combination of commercial detergent and biodetergent was the second highest in achieving a good performance of washing. The component inside commercial detergent that added with the enzyme from the biodetergent has helped in improving the washing performance compare to the biodetergent itself. However, the performance of Rhamnolipid NR22 cannot be denied as an excellent potential Green Alternative. It showed the efficiency almost similar to the efficiency of commercial detergent in the market.

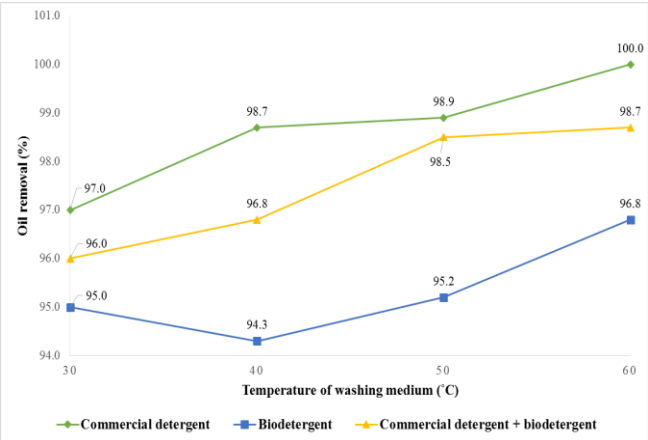

Fig. 10: Effect of temperature on the removal of oil from cotton cloth. pH = 7; stirrer velocity = 500 rpm; washing time = 20 min

E. SEM of treated cotton cloth

Figure 11A shows the representative SEM image of untreated cotton cloth while Figure 11B represented the image of cotton cloth after being washed by using biodetergent. The surface of the cotton cloth A was covered with oil at some parts of the fabric. The images of the cotton cloth before washing indicated that the fabric was dark in colour compared to the cotton cloth of Figure 11B which appeared brighter. Furthermore, it looks untidy and a bit messy in the appearance of the cotton cloth. This could be concluded that biodetergent have the capability and high potential in acting as a good surfactant and detergent in order to reducing the used of commercial detergent that may bring harms and problems towards the environmental.

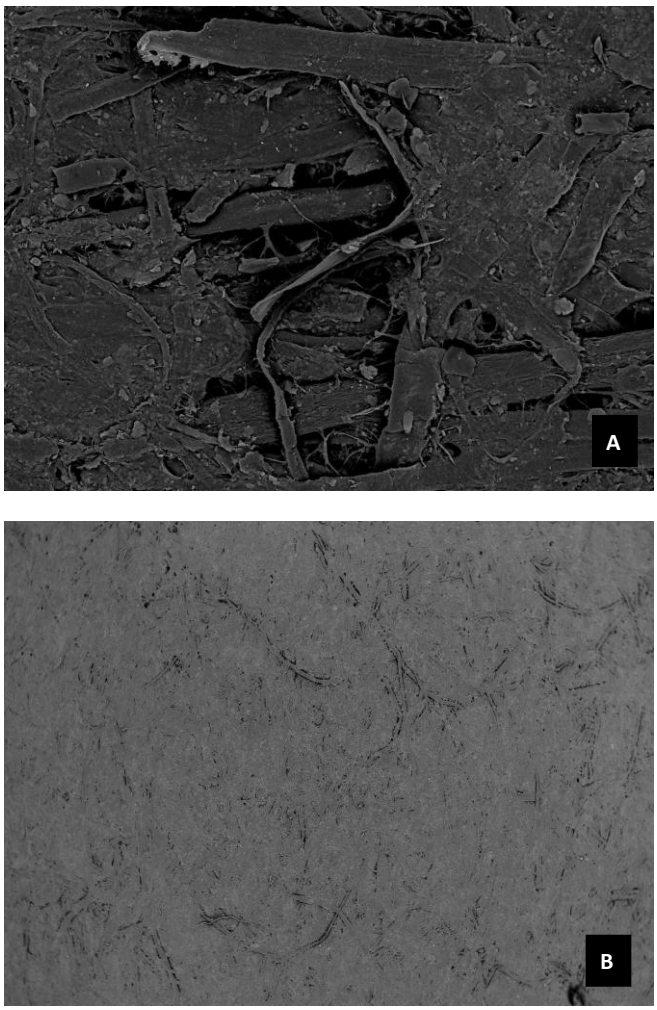

Fig. 11: Representative SEM images of (A) untreated cotton cloth and (B) treated cotton cloth after being washed by using Rhamnolipid NR22.

IV. CONCLUSION

Rhamnolipid NR22 has showed green detergent ability. The production of Rhamnolipid NR22 was important to make sure its components have the ability to operate the same mechanism with commercial detergent. A testing of FTIR has been done to compare the chemical structure of the Rhamnolipid NR22 with the commercial biosurfactant. For the washing efficiency by using 3 types of samples, commercial detergent was proved to be the highest in percentage of oil removal since the formulation of the detergent itself has been modified. Nevertheless, the efficiency of biodetergent rhamnolipid achieved an excellent result too since the value of oil removal percentage was nearly similar to the commercial detergent. Washing performance in different temperatures shows that as the temperature of washing medium increasing, the percentage of oil removal also increasing. However, the ability of being one the good surfactant of rhamnolipid NR22 cannot be denied when SEM images shows a clearly difference of cotton cloth before and after washed with biodetergent.

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