BIO-SURFACTANT SYNTHESIS FROM WASTE COOKING OIL

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BIO-SURFACTANT SYHNTHESIS FROM WASTE COOKING OIL

NUREDAH AISYAH BINTI NOR AZMAN

Thesis submitted in partial fulfilment of the requirements
for the award of the degree of
Bachelor of Chemical Engineering (Pure)

Faculty of Chemical & Natural Resources Engineering
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SUPERVISOR’S DECLARATION

We hereby declare that we have checked this thesis and in our opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Bachelor of Chemical Engineering (Pure).

Signature	:
Name of main supervisor	: DR. IR. SAID NURDIN
Position	: SENIOR LECTURER
Date	:

STUDENT'S DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged. The thesis has not been accepted for any degree and is not concurrently submitted for award of other degree.

Signature :
Name : NUREDAH AISYAH BINTI NOR AZMAN
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Dedication

To my parents,

To my brothers and sisters,

Thank you for being understanding, supportive,
motivating, inspiring and giving me strength.

ACKNOWLEDGEMENT

In the name of Allah, the most merciful.

Thanks to Allah S.W.T for his gracious and merciful in giving me strength, guidance and opportunity to complete this project.

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Thanks to my parents for their motivation, ideas, inspiring, encouragement and financially during my year of studies leading to the completion of this project. Besides, thanks to my brothers and sisters for their opinion and ideas for completing this project.

Special thanks to my lecturers, dearest friends and teammates and others that have been contribute directly or indirectly and helping me to complete this project paper.

ABSTRACT

This paper presents the bio-surfactant synthesis from waste cooking oil using alkaline base. Sodium hydroxide will be used as alkaline base. Waste cooking oil is chosen as raw materials in synthesizing bio-surfactant due to fatty acid content and this non-edible oil can be used as an alternative raw. Nowadays, the surfactants mostly made- up from non- renewable sources. The majority of the currently used surfactants are petroleum-based and are produced by chemical means. Waste cooking oils are waste and unuseful material. Making bio-surfactant using waste cooking oil which is inedible vegetables oil versus the human food chain. The mains purpose of this study are to synthesize bio-surfactants from waste cooking oil using base to examine the parameters effect on the bio-surfactant yield. The oil will be added with NaOH and the mixture will be stirred continuously at certain temperature. Then, sulphuric acid and hydrogen peroxide will be added. Several of process temperature, time and concentration of NaOH will be examined. This can improve the yield of bio-surfactant. Furthermore, the process parameter affects the different maximum yield of the bio-surfactants. The resulting bio-surfactant was washed with concentrated sodium chloride, let stand, filtered, washed with distilled water twice and dried in the oven at 60 °C for 24 hours. Then, the characteristic of the bio-surfactant was analyzed and compared with the commercialized detergent. The pH, interaction with hard water, emulsification with oil and height of foam fulfilled the set criteria by ASTM D460.

Key words: biosurfactants, waste cooking oil, sodium hydroxide

ABSTRAK

Kertas kerja ini membentangkan sintesis bahan pencuci bio daripada sisa minyak masak menggunakan asas alkali. Natrium hidroksida akan digunakan sebagai asas alkali. Sisa minyak masak dipilih sebagai bahan mentah dalam sintesis bahan pencuci bio kerana kandungan asid lemak dan minyak yang tidak boleh dimakan ini boleh digunakan sebagai alternatif mentah. Pada masa kini, bahan pencuci bio kebanyakannya diperbuat daripada sumber yang tidak boleh diperbaharui. Majoriti bahan pencuci kini digunakan adalah berasaskan petroleum dan dihasilkan dengan cara kimia. Minyak masak adalah sisa buangan dan bahan yang tidak berguna. Membuat bahan pencuci bio menggunakan sisa minyak masak yang merupakan minyak sayur-sayuran boleh dimakan berbanding rantaian makanan manusia. Tujuan utama kajian ini adalah untuk membuat bahan pencuci bio daripada sisa minyak masak menggunakan asas untuk mengkaji kesan parameter pada hasil bahan pencuci bio. Minyak ini akan ditambah dengan NaOH dan campuran akan dikacau secara berterusan pada suhu tertentu. Kemudian, asid sulfurik dan hidrogen peroksida akan ditambah. Beberapa proses suhu, masa dan kepekatan NaOH akan diperiksa. Ini boleh meningkatkan hasil produk bahan pencuci bio. Tambahan pula, proses parameter yang berlainan mampu memberikan kesan maksimum kepada produk. Yang terhasil bahan pencuci bio telah dibasuh dengan natrium klorida pekat, dibiarkan, ditapis, dicuci dengan air suling dua kali dan keringkan di dalam ketuhar pada suhu 60 ° C selama 24 jam. Kemudian, sifat bahan pencuci bio dianalisis dan dibandingkan dengan bahan pencuci komersial. PH, interaksi dengan air keras, pengemulsian dengan minyak dan ketinggian buih memenuhi kriteria yang ditetapkan oleh ASTM D460.

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1 INTRODUCTION

1.1 Background of study

Biosurfactants or others name are surface active agents are friendly environmental surfactants. Nowadays, biosurfactants become most important surfactant because of their benefit. Surfactants constitute an importance class of industrial chemicals. The industrial demand for surfactants has grown to about 300 % within the US-chemical industry during the last two decades (Cameotra,2010).

Biosurfactants are amphiphilic biochemical compounds. Most of them are produced by microbial cell surfaces. Amphiphilic structure have ability to reduce the interfacial energy. (Adria et al, 2003; Yagnik, 2013; Fakruddin, 2012). Biosurfactants have two different group which are hydrophilic head and hydrophobic tail. According to the Kopeliovich (2013), hydrophilic head are polar and as we know, polar will attract the polar solvents and mostly water. Water has positively charged which is Hydrogen atom and negatively charged which is Oxygen atom. Hydrophilic head also known as lipophobic. This because oil molecules which is non-polar molecules will repel with the polar heads. While hydrophobic tails are non-polar and they will repel with the polar molecules. Hydrophobic tails also are lipophilic because they have affinity with the fats (Kopeliovich *et al*, 2013).

Nowadays, there are many ingredients for synthesizing bio-surfactants. The majority of the currently used surfactants are petroleum-based and are produced by chemical means. These compounds are often toxic to the environment and may affect the ecological problems particularly in washing applications as these surfactants inevitably end up in the environment after use (Navare,2013).Current surfactants are synthesized by using linear chain alkylbenzene sulfonate or branched chain alkylbenzene sulfonate(Ameh *et al*, 2010).Unfortunately, this synthesis cause environment effect. This because of straight chain surfactant did not work in hard water. To overcome this problem, phosphate will be added to soften the water. Unfortunately, algae food's is phosphate and it can growth in seas and river. The algae will decrease the oxygen level in water and will destroying the aquatic life. This also will affect the habitat in seas or river. While for branched chain molecules, microbes are not able to break down branch chain. This problem will make foam to be left on surface of the water (Kohler,2006).

To overcome this problem, there are too many biosurfactants synthesis were produced. Most of the biosurfactants are made up from renewable resources, eco-friendly and non- toxic. Other than that, they also synthesizing biosurfactants from vegetable oil. Nowadays, there are too many research and product of biosurfactants synthesis using vegetable oil such as from waste cooking oil, sugarcane, sunflower oil, soybean oil, palm oil, jatropha oil, waste cooking oil and also from castor oil (Kasturi *et al*, 2013). According to Saeesh (2014), waste cooking oil also can form surfactant.

In a modern society, oil is commonly used for the preparation of food. In today's world, oil frying method is widely used due to the contribution of good taste, attractive color and better presentation of the food. As this method becomes increasingly popular, accumulation of waste generated from cooking oil also increases. In 2008, Malaysia produced 17.7 million tons of palm oil on 4.5 million hectares of land and was the second largest producer of palm oil, is employed more than 570,000 people (Global Oils & Fats Business,2008;Szmigielski,2008). Malaysia is the largest exporter of palm oil in the world. Most cooking oil made from palm oil. The local disposal of cooking oil becomes a huge problem because of the large volumes involved. In the fast food business alone, a single branch which serves fried food such as fried chicken, french fries and burgers can produce as much as 15 liters of used cooking oil per day. Considering that there are hundreds of these outlets in Malaysia, the total amount generated can reach several thousand liters per day. Properties of degraded used cooking oil after it gets into sewage system are conductive to corrosion of metal and it also affects installations in waste water treatment plants. Thus, it adds to the cost of treating effluent or pollutes waterways. According to Lai (2014), Sekitar Bumi Hijau Sdn Bhd, the collected waste cooking said that over 100 tonnes of used cooking oil in 2011, 192.82 tonnes in 2012 and more than 200 tonnes last year.

Furthermore, by using waste cooking oil, severe environmental problems can be avoided because a liter of oil poured into a water course can pollute up to 1000 tanks of 500 liters (Carlos *et al*, 2011), it's feasible to demonstrate the contamination with the dumping of these oils to the main water sources. The oil which reaches the water sources increases its organic pollution load, to form layers on the water surface to prevent the oxygen exchange and alters the ecosystem. The dumping of the oil also causes problems in the pipes drain obstructing them, creating odors, increasing the cost of wastewater treatment and can clog pipes and damage wastewater or septic systems.

The replacement of the main ingredients of surfactant with waste cooking oil is to make a safe and environmental friendly surfactant as well as reducing the feedstock cost and that the bio- surfactant are biodegradable.

1.2 Motivation

Bio surfactants is becoming an important product in chemical industry. This appearing in essential biological systems and industrial process example like lubricants, laundry surfactant, dishwashing liquid, shampoos, soaps, mining flocculates, wastewater treatment, textile processing and many other products and processes. In 2010, about $24.3 billion get from worldwide market for surfactant only and about 10 million metric tons were produced (Hayes, 2012).

Bio surfactant are beneficial to environment because non-toxic, biodegradable and eco-friendly are very useful. Bio surfactants can avoid contamination and safe to animals, humans and environment. The advantages of bio surfactants are lower toxicity, higher biodegradability, higher specificity and effectiveness for pH values and at extreme temperature (Cipinyte *et al*, 2011;Chomiczkewska *et al*, 2011;Mensah *et al*, 2011).

In Malaysia, the exposure about the advantage of waste cooking oil is still low. Most Malaysian will throw the cooking oil into drain and causes problems in the pipes drain obstructing them, creating odors, increasing the cost of wastewater treatment and can clog pipes and damage wastewater or septic systems. Because of the large amounts of waste cooking oil in Malaysia,we want to make the oil useful too in Malaysia. We want to make biosurfactant from the waste cooking oil.

1.3 Problem statement

Some of surfactant were made up from non-renewable and mostly are with toxic, non-biodegradable and not eco-friendly to surrounding and environment. This will cause problem to human, animals and environment. Some surfactant that is poor biodegradability mostly under anaerobic condition may cause result in high surfactant sludge load (Ameh *et al*, 2010).Furthermore, in Malaysia, waste cooking oil are waste and unuseful material. Making waste cooking oil as useful material can avoid many problem. Edible vegetables oil not only for human food chain but also for bio surfactant synthesis.

1.4 Objectives

The main objectives or this research are:

1. To synthesize bio surfactants from waste cooking oil using base process.
2. To examine the parameters effect (concentration of NaOH and enzyme, time and temperature) on the bio surfactants yield.

1.5 Scopes

Four scopes of study have been determined in this study that is:

1. Treatment of waste cooking oil involving filtration.
2. Synthesis of bio surfactants from waste cooking oil using base process.
3. Variation of process parameter (temperature, time, concentration of NaOH will be examined) for bio surfactant synthesis.
4. The physical and chemical properties of bio surfactants will be analyzed.

2 LITERATURE REVIEW

2.1 Bio surfactants

Biosurfactants are amphiliphic biological and surface active compounds produced by microorganisms. They have different structures including glycolipids, lipopetides, polysaccharide proteins, phospholipids and fatty acids. Most of them are produced by microbial cell surfaces. Amphiphilic structure have ability to reduce the interfacial energy. (Bodour *et al*, 2003; Yagnik *et al*, 2013; Fakruddin, 2012). Bio-surfactants can reduce surface and interfacial tension. The amphiphiles that form micelles can be potentially used for surface chemical works. All surfactant have two ends namely a hydrocarbon part which is less soluble in water and the water soluble. Hydrophobic end are less soluble in water and the molecule is a long-chain of fatty acids or hydroxyl fatty acids. The hydrophobic moiety is usually a C8 to C12 alkyl chain or alkyl aryl that may be linear or branched. While for hydrophilic which is soluble in water can be a carbohydrate, amino acid, cyclic peptide, phosphate, carboxylic acid or alcohol. (Suresh *et al*, 2012).

Biosurfactants also can be produced by several types of microorganisms such as fungi, bacteria and yeasts. Bacillus strain can produced lipopeptide biosurfactants likes Bacillomycin F,D,L, surfactin, iturin, lichenysin, halobacilin and plipastatin. Among them, surfactin are the most effective biosurfactants. This biomolecule is usually a cyclic compound consisting of seven amino acids bonded to a lipid moiety. Surfactin also effective in lowering the surface reaction of water to < 30 mN/m which is comparable with the values obtained by conventional synthetic surfactants (Bahry *et al*, 2013). The charcteristic of surfactin are antibiotic and antiviral properties. Hydrocarbons are commonly used as the substrate for production of biosurfactants. biological function of surface-active compounds is related to hydrocarbon uptake and a spontaneous release occurs with this subtrates (Namir *et al*, 2009).

Futhermore, biosurfactant synthesis are from renewable resource. The biosurfactant can be made from sodium or potassium salt too. But the fatty acid from soap are less soluble compared to sodium or potassium salt. The effect will be formation precipitate or soap scum. The reaction between fats and alkali base can form biosurfactant. To be qualified as a raw material for biosurfactant synthesis, there must presence of triglycerides in selected vegetable oil. A good biosurfactant must have lower toxicity, higher biodegradability, higher specificity and effectiveness for pH values and at extreme temperature (Fakhruddin,2012).

According the Fakhruddin (2012), there are few factor that will affect the biosurfactant production. The factor are carbon sources, nitrogen sources, environmental factors, aeration, agitation and lastly, salt concentration. The nature of the carbon substrate will affect and influence the quality and quantity of biosurfactant production. Crude oil,diesel, glucose, sucrose glycerol have been reported to be a good source of carbon substrate. Nitrogen is a important for microbial growth as protein and enzyme syntheses depend on it. Example of nitrogen for biosurfactant synthesis are urea, peptone, ammonium sulphate, yeast extract, ammonium nitrate, meat extract, malt extracts and sodium nitrate. Among them, yeast extract is the most used nitorgen source and its usage for concentrtion is organism and culture medium dependent. Most bio surfactant productions are reported to be performed in a temperature range of 25-300 °C. The effect of pH on bio surfactant production occurred when the pH was 8 which is the natural pH of sea water. Aeration and agitation function as facilitate the oxygen transfer from the gas phase to the aqueous phase in the production of bio surfactants. Furthermore, the production of bio emulsifiers can enhance the solubilization of water insoluble subtrates and consequently facilitate nutrient transport to microorganism. According to the observation, the best production value occur when the air flow rate was 1vvm and the dissolved oxygen concentration was maintained at 50% of saturation. Salt concentration also important because it affect the cellular activities of microorganisms. Based on observation, some bio surfactant products which were not affected by concentrations up to 10% although slight in the Critical Micelle Concentration (CMC) were detected (Fakruddin,2012).

There are several properties of bio surfactant. One of it are surface interface activity. A good surfactant has lower surface tension range between 72 to 35 mN/m and the interfacial tension of water, hexadecane from 40 to 1 mN/m (Mehta *et al*,2010). Bio surfactant are more effective and efficient and their CMC is about 10 to 40 times lower than that chemical surfactants. Many bio surfactants and their surface activities are undeterred under environmental stress such as temperature and pH. The surface activity did not changed even pH 5 to 11. Different with synthetic surfactants, microbial-produced compounds are easily degraded and particularly suited for environmental applications. From six bio surfactants which are four synthetic surfactant and two commercial disprersants, the most biosurfactants can degraded faster except for synthetic sucrose-strerate. It showed structure homology to glycolipids and was degraded more rapidly than the biogenic glycolipids (Yagnik *et al*,2013).

For a good bio- surfactant, the basic properties that it must achieve are foamability, economic, non-corrosive, interaction with oil, non-toxic and high biodegradability and interaction in hard water.

Below shows the physicochemical analysis of bio- surfactant:

a. Foamability

b. Non-corrosive

c. Interaction with oil

d. Interaction in hard water

Properties	Soap	Surfactant
pH	Slightly alkaline	Can be controlled to suit the cleaning task
Formation of scum	Form scum in hard water	Does not form scum in hard water
Cleaning power	Less effective	More effective
Ease of rinsing	Difficult to wash all soap on clothes. The soap that remains leave an odor and spoils the fabrics	Rinse out well from clothes

Table 1: Difference between soap and surfactant

2.2 Raw materials

Cooking oil in Malaysia usually from palm oil. About 80% of the national production of crude palm oil is used for food purposes, mainly as cooking oils. The CPO produced by the mills have to be refined to meet the industries and international standards (FAO's Codex Alimentarius) for edible oils. The production of refined oil is undertaken in 57 refineries in Malaysia with a total refining capacity of 15.5 million tonnes CPO per year (Hai,2006).

Refined palm oil and palm olein recommended for cooking and frying oil due to their good resistance to oxidation at frying temperature which is very high temperature. Palm oil contain fatty acids,fatty ester and fatty alcohols which are suitable to make diesel, flavor and fragrance industries, the production of candles and soap, as processing aid for rubber products and as active ingredient for washing and cleaning products (History Of The Malaysian Palm Oil Industry,2013).

Mostly waste cooking oil is generated from fried food like chicken fried, which need a large amount of oil to full immersion of food. The temperatures are greater than 180 °C. Because of high temperatures, it will changes the chemical and physical composition (Carlos *et al*, 2011). In the fast food business alone, a single branch which serves fried food such as fried chicken, french fries and burgers can produce as much as 15 liters of used cooking oil per day. Considering that there are hundreds of these outlets in Malaysia, the total amount generated can reach several thousand liters per day.

Used cooking oil is normally black, a strong odor and does not have large amount of solids because its collection is passed through a fine mesh. Usually, most private households dispose their cooking oil by flushing it down the sink. This may block drains and cause the sewerage not to be used by catching other waste materials in the sewerage system. The effect may damage the waste water treatment plants and raise processing costs. Fat covering elements of installations and therefore reducing flow in them decreases their efficiency. About 40% of the sewerage system blockages are caused by the waste frying oil poured into sink. Furthermore, waste frying have eco-toxic properties which mean if they are spilled onto ground, the soil will be contaminated and damage the plants (Sanli *et al*, 2011). Even a tiny amount of oil or grease can affect the bacteria that makes a septic tank work, causing major and expensive problems. Nevertheless, fat from waste cooking oil is a rat's favourite food and can attract them to a drain and can cause disease (Michelle,2011).

Figure 1: Waste cooking oil

To avoid problem in sewerage system, waste cooking oil should be recycled back. Waste cooking oil can be recycled to become biodiesel, strengthen nails, skin moisturizer, hair care, restoring glow of utensils, adhesives remover and for making soap and surfactant like shown in Figure 1.

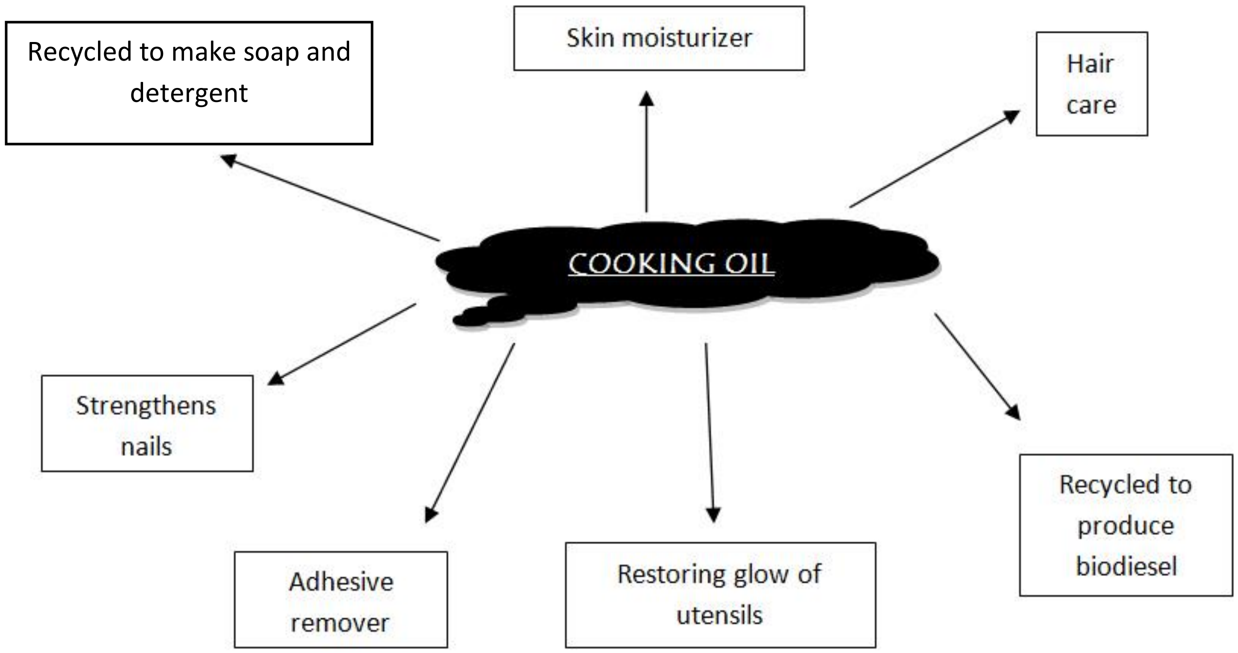


Figure 2: Useful of waste cooking oil

PRODUCT	**PARAMETER**	**SPECIFICATION**
RBD Palm Oil	FFA (as palmitic)	0.1% max
	M&I	0.1% max
	IV (Wijs)	50 – 55
	SMPT (AOCS Cc 3-25)	33 – 39
	Color (5¼ Lovibond cell)	3 Red max

Figure 3: Product specification of cooking oil *(Delima Oil Products Sdn Bhd,2013)*

3 MATERIALS AND METHODOLOGY

3.1 Introduction

Waste cooking oil were collected at Restaurant Nasi Kukus Mama in Gambang, Pahang. The chemical used to biosurfactants are sodium hydroxide, sulphuric acid, hydrogen peroxide, sodium chloride and magnesium sulphate.

3.2 Materials

I. Waste cooking oil
II. *Sodium hydroxide*
III. *Sulphuric acid*
IV. *Hydrogen peroxide*
V. *Sodium chloride*
VI. *Magnesium sulphate*

3.3 Apparatus

I. Reagent bottle
II. Hot plate with magnetic stirrer
III. Separator funnel
IV. Beaker
V. Conical flask
VI. Test tube and stopper
VII. Measuring cylinder
VIII. Volumetric flask
IX. Pippete
X. Burette
XI. pH paper
XII. Retort stand

3.4 Overall Methodology

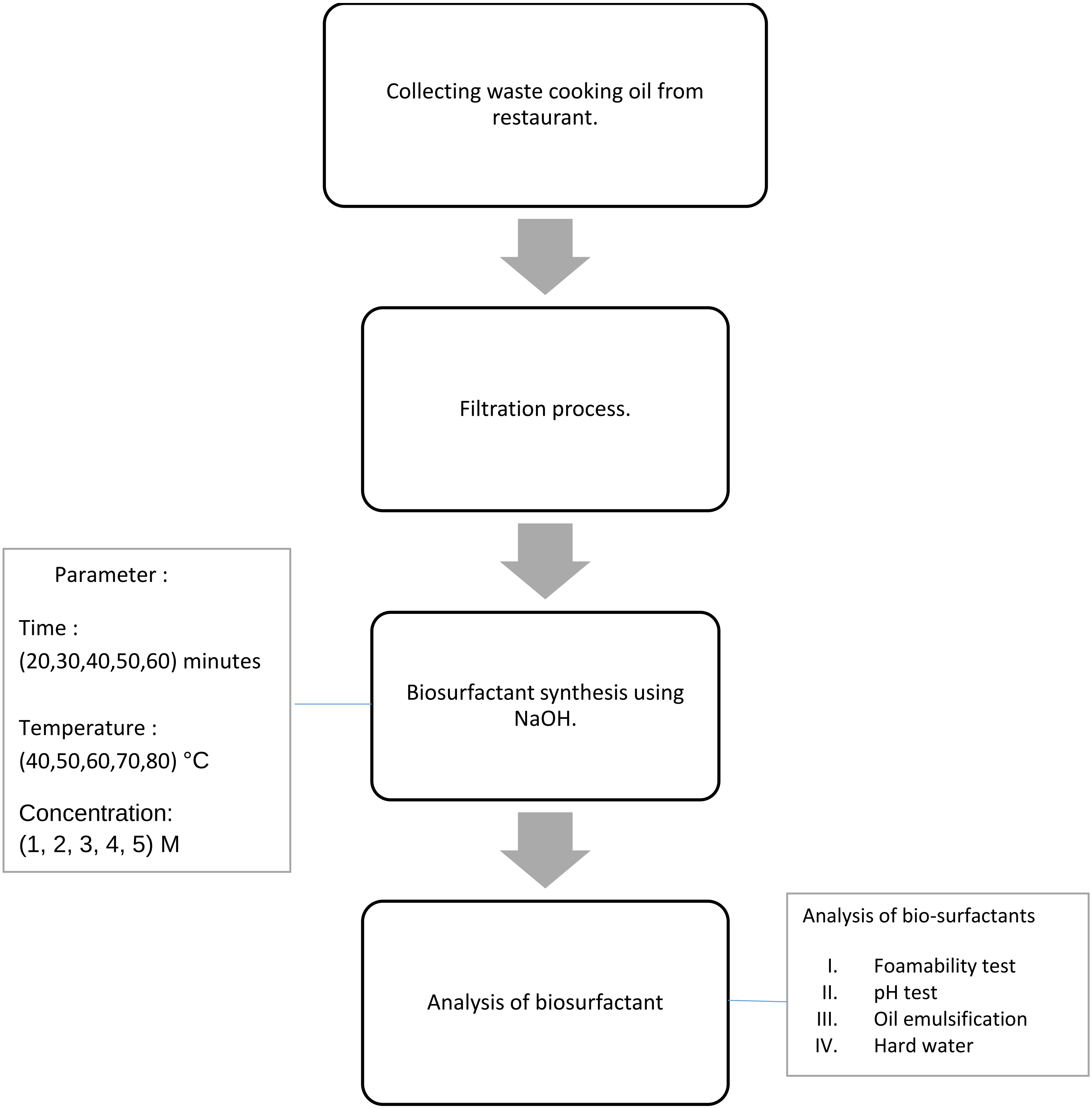


Figure 4: Overall flow chart of bio surfactants synthesis

3.5 Experimental methodology

3.5.1 Pre- treatment of waste cooking oil

Waste cooking oil will be collected at Restaurant Nasi Kukus Mama, Gambang, Pahang. For treatment of adequacy of waste cooking oil, the operations that can be applied is filtration. Degumming and deodorization aren't needed because the oil have been treated prior to use and although during degradation odors occur, the removal is not essential for the biosurfactant production. Function of filtration is for removing solid, inorganic material and other contaminants in the oil. First, waste cooking oil will be heated at 60 °C, because the substances carbonaceous produced from burnt organic material and can remove solid fats or products of low melting points from the frying process. After that, let it cooled at room temperature. Waste cooking oil are ready to be used for bio-surfactant synthesis (Carlos *et al*,2011).

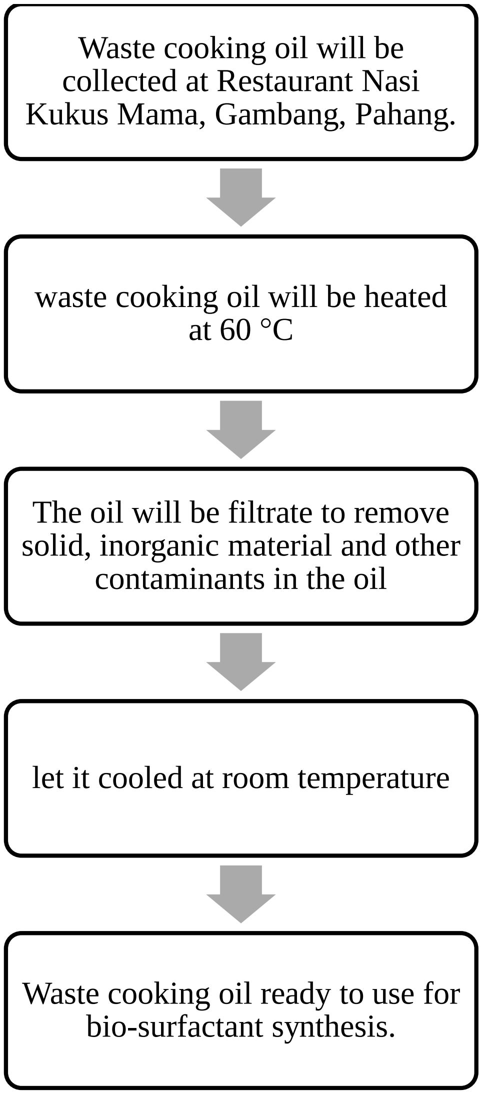
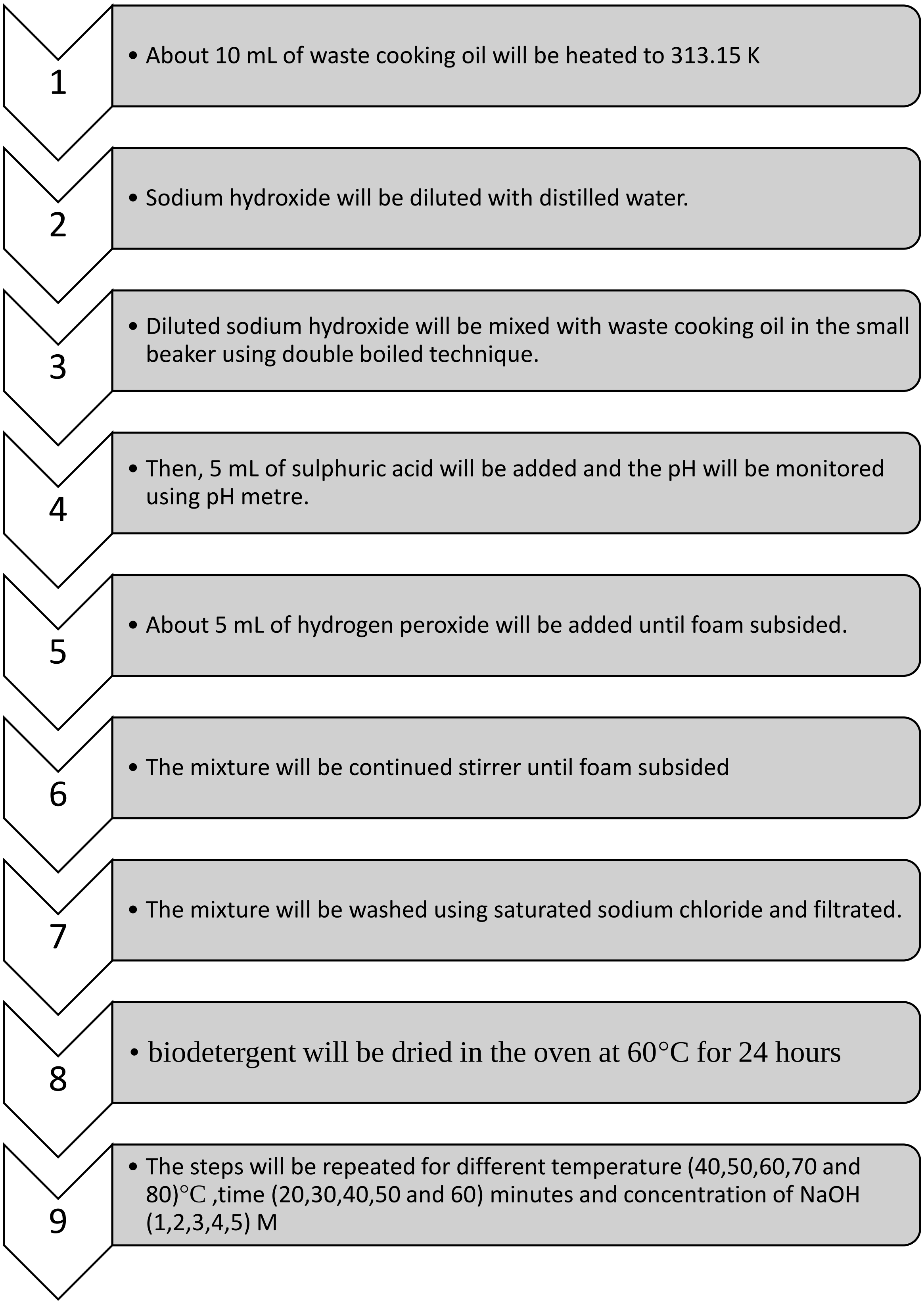


Figure 5: Flow diagram for waste cooking oil treatment

3.6 Synthesis of bio-surfactants

3.6.1 Synthesis of bio-surfactants using sodium hydroxide

About 10 mL of waste cooking oil will be heated to 313.15 K. Sodium hydroxide solution will be diluted with distilled water and mixed with waste cooking oil. The mixture will be stirred using magnetic stirrer with hot plate. Then, 5 mL of 3M sulphuric acid will be added and the pH will be monitored using pH metre .About 5 mL of hydrogen peroxide will be added until foam subsided. The mixture will be continued stirrer until foam subsided. Then, the mixture will be washed using saturated sodium chloride, filtrate and dried in the oven at 60 ℃ for 24 hours. The steps will be repeated for different temperature (40,50,60,70 and 80) ℃,time (20,30,40,50 and 60) minutes and concentration of NaOH (1,2,3,4,5) M.



1
- About 10 mL of waste cooking oil will be heated to 313.15 K

2
- Sodium hydroxide will be diluted with distilled water.

3
- Diluted sodium hydroxide will be mixed with waste cooking oil in the small beaker using double boiled technique.

4
- Then, 5 mL of sulphuric acid will be added and the pH will be monitored using pH metre.

5
- About 5 mL of hydrogen peroxide will be added until foam subsided.

6
- The mixture will be continued stirrer until foam subsided

7
- The mixture will be washed using saturated sodium chloride and filtrated.

8
- biodetergent will be dried in the oven at 60°C for 24 hours

9
- The steps will be repeated for different temperature (40,50,60,70 and 80)°C ,time (20,30,40,50 and 60) minutes and concentration of NaOH (1,2,3,4,5) M

Figure 6: Flow chart of bio-surfactants synthesis using NaOH.

3.6.2 Bio-surfactants analysis

3.6.2.1 pH test

The purpose of pH test is controlled in a range that will not be harmful for the users. About 2 g of biosurfactant will added into test tube. 100 ml of distilled water will be added the mixture will be stirred using glass rod. The pH paper will be touched to the glass rod. Compared the pH color with others commercialized detergent.

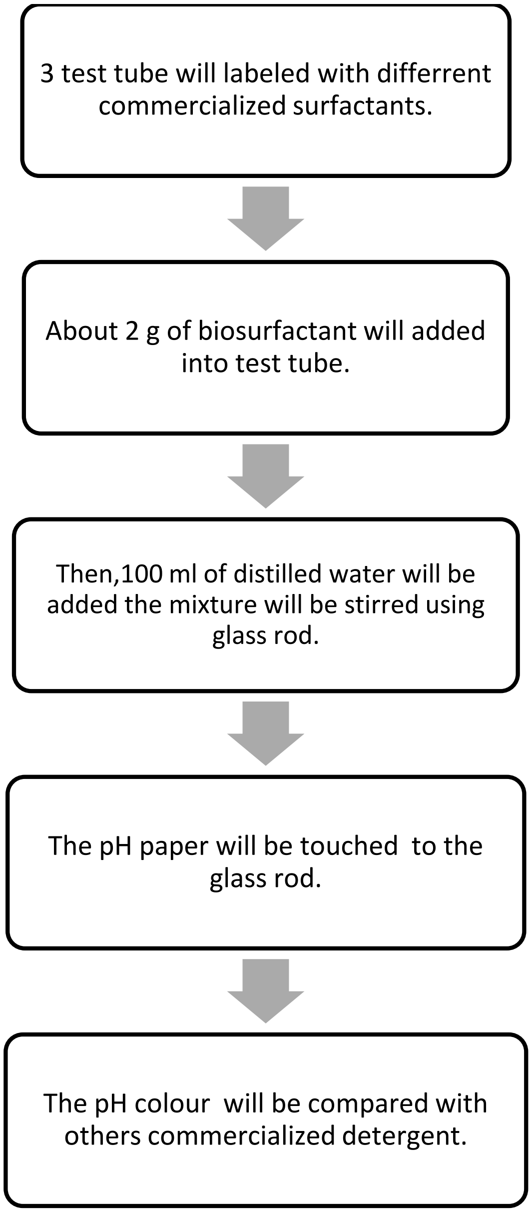


Figure 7:Flow chart for pH test.

3.6.2.2 Foamability test

For foamability test, 2 g of bio surfactants will be mixed with 100 ml of distilled water into test tube with stopper. The mixture will be shacked vigorously and leaved for 2 minutes and 10 minutes. The height of the foam will be measured. The step will be repeated using others commercialized surfactants.

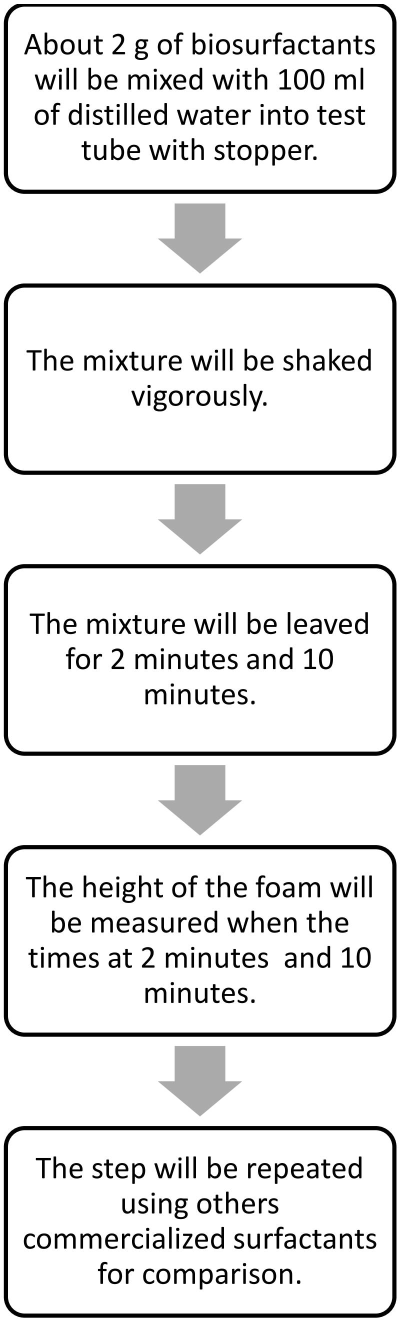


Figure 8:Flow chart for foamability test.

3.6.2.3 Hard water

Hard water will be prepared by adding 100 mL of distillate water and 2g of magnesium sulfate, $MgSO_4$. The mixture will be stirred. For comparison, half-fill the test tube will be added with tap water then about pea-sized of surfactant will be added to the both test tube. The test tube will be shacked vigorously to take the reading of height of the suds in both test tube. Compared the height of foam from foamability test with height of foam from hard water.

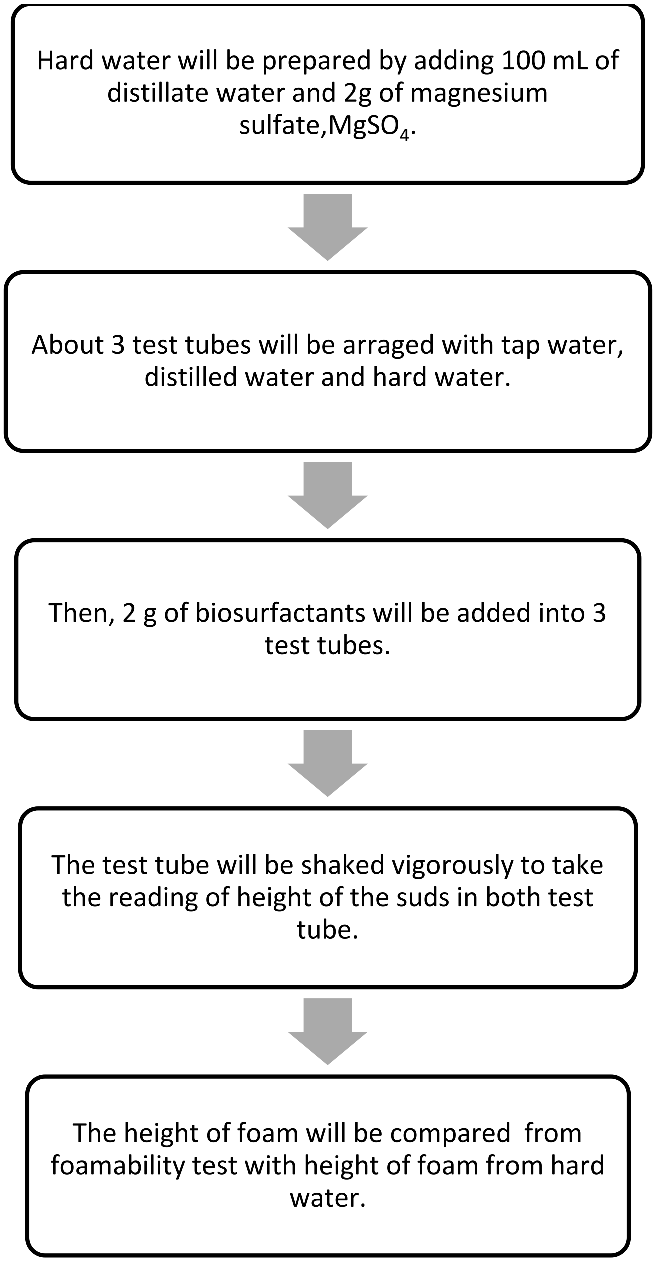


Figure 9: Flow chart for hard water test.

3.6.2.4 Oil emulsification

About 4 drop of cooking will be added into the test tube. Then, 5 mL of the bio surfactant will be added into the same test tube. The test tube will be shacked vigorously and leaved for 2 minute and 10 minutes. Repeat the step by replacing the bio surfactant with others commercialized surfactants. Then the height of foam produced will be recorded.

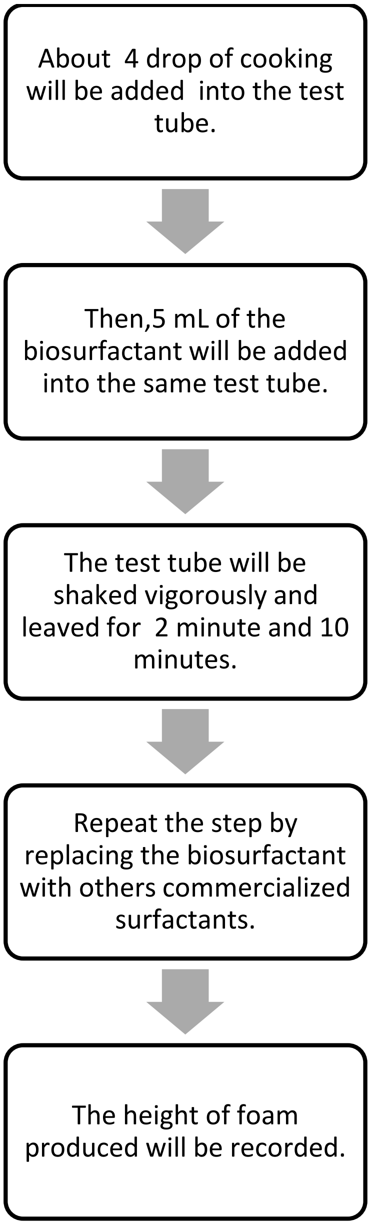


Figure 10: Flow chart for oil emulsification.

4 RESULT AND DISCUSION

In this research, experiments were carried out by batch method to synthesis bio- surfactant using waste cooking oil in order to identify the effect of variation of process parameter such as time, temperature and concentration of alkali base that would give improvement on the efficiency of bio surfactant yield. The percentage yield of bio- surfactant can be calculated and plotted by using the formula below.

$$\% \, Yield = \frac{actual \ yield}{theoretical \ yield} \times 100 \ \%$$

Equation 1: Percentage yield formula

4.1 Preparation of waste cooking oil

Waste cooking oil will be collected at Restaurant Nasi Kukus Mama, Gambang, Pahang. For treatment of adequacy of waste cooking oil, the operations that can be applied is filtration. Degumming and deodorization aren’t needed because the oil have been treated prior to use and although during degradation odors occur, the removal is not essential for the bio- surfactant production. Function of filtration is for removing solid, inorganic material and other contaminants in the oil.

4.2 *Effect of Concentration on Bio- surfactant Yield*

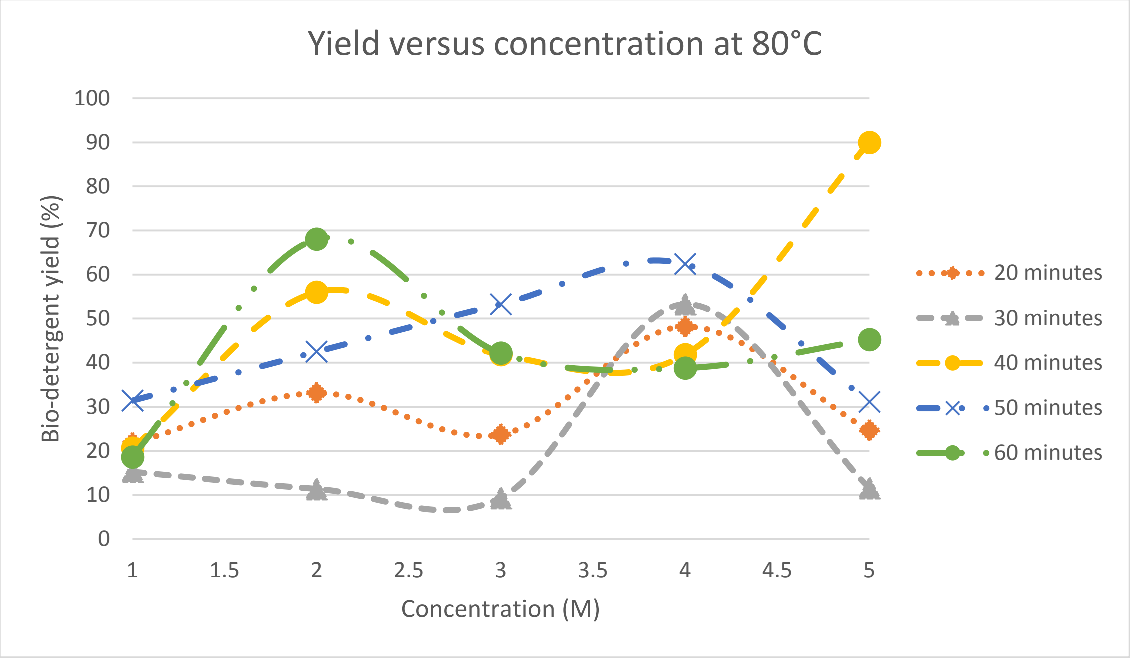


Figure 11: Yield versus concentration at temperature 80°C

Figure 11 above shows the effect of alkali base concentration on the yield of bio surfactant at constant time. Each sample was run in a same process with constant amount of oil at difference concentration and time. The yield was considered from the weight of bio surfactant produced. Based on Figure 11, at 20 minutes, the yield is fluctuated. Its start increasing at 2 M then decreasing about 23.69% at 3 M, increasing back at 4 M which is the highest percentage yield produced and dropped to 24.62% at 5 M.

At time 30 minutes, the yield start with 15.24 % at 1 M then keep decreasing at 2 M and 3 M. The yield shoot up increasing at 4 M which is the highest yield for 4 M about 53.26 %. At 5 M, yield decreased to 11.54%. According to Wish (2009), low concentration of hydroxide indicates insufficient hydroxide. Thus many fats will remain in the mixture causing it to separate instead of forming a bio surfactant.

At time 40 minutes, the yield start increasing at 1 M and 2 M. Yield become constant for 3 and 4 M. The yield starts to increase tremendously reaching 90% at 5 M which is the highest yield production. Saponification value and type of the oil used effect the concentration of hydroxide. This proved that higher concentration of hydroxide gave off higher surfactant yield. Unfortunately it will leave the surfactant having higher pH (Kevin,2008).

At time 50 minutes, the yield start increasing from the beginning until the concentration increases up to 4 M and fall back at 5 M. Start from 1 M, the yield is 31.35 % and keep increasing until 62.36% at 4 M which is the highest yield. Yield dropped to 31.08% at 5 M. If concentration of alkali is too high, water to react will be less and saponify the oil. Finally, formation of surfactant will be stopped (Wish,2009).

At time 60 minutes, the highest yield at 2 M and the lowest yield at 4 M. The yield of bio surfactant start increasing from the beginning at 18.62% to 68% the suddenly dropped 42.15% at 3 M and keep decreasing to 38.77% at 4 M. At 5 M, yield increased significantly to 45.23%.

4.3 Effect of Time on Bio Surfactant Yield

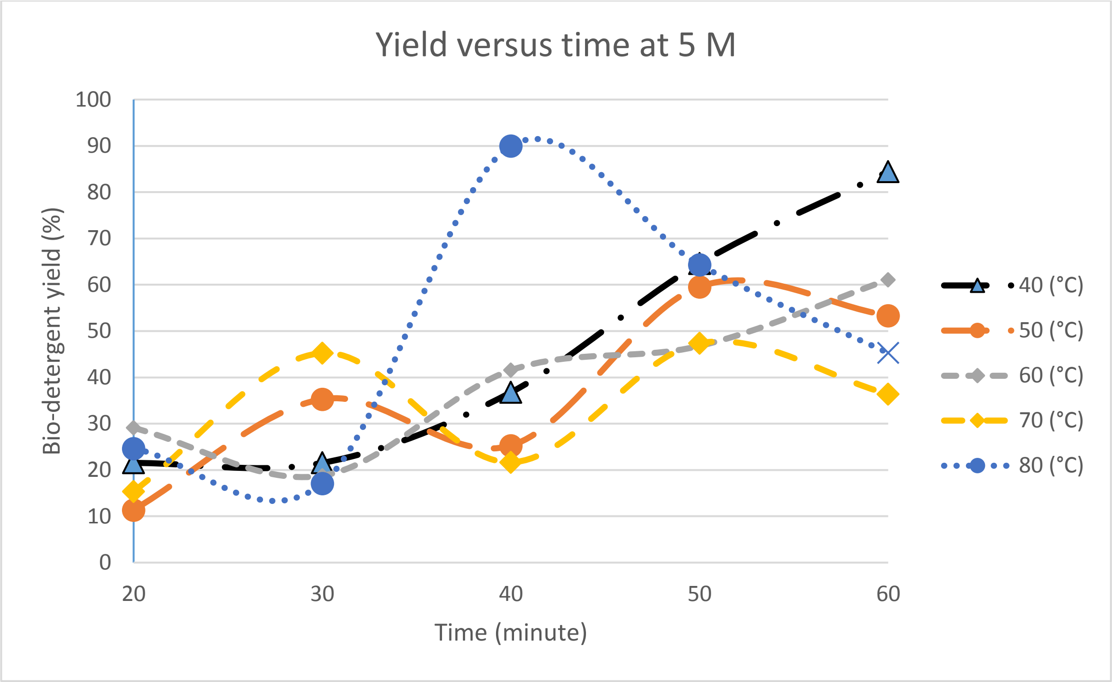


Figure 12: Yield versus time at concentration 5 M

From Figure 12, it shows the effect of time on the yield of bio surfactant at constant concentration. Each sample was run in a same process with a constant amount of oil at difference temperature and time. At 40 °C, bio surfactant yield was constant by 21.53% for 20 and 30 minutes. After 30 minutes, yield rose increasingly and grew rapidly to highest yield at 60 minutes from 21.53% at 30 minutes to 36.77% at 40 minutes then keep increasingly to 64.62% at 50 minutes and grey rapidly to 84.46% at 60 minutes.

At 50 °C, it show that the amount of bio surfactant produced was inconsistent and kept on fluctuating. The yield start with 11.35% for 20 minutes and increased steadily to 35.25% for 30 minutes. But suddenly declined dramatically to 25.27% at 40 minutes. Yield increased rapidly to 59.67% which the highest yield at 50 minutes.

At 60 °C, the yield start at 29.07% at shorter process time and rose slowly when the process time is longer which is from 30 to 60 minutes. At 70 °C, the yield was fluctuating. The yield start to increase from 20 minutes to 30 minutes. From 30 minutes, yield dropped slowly to 40 minutes and rose for a short time then declined when the process time is longer.

At 80 °C, a slight drop in the yield can be observed at the start of the experiment. The yield started to grew greatly at 40 minutes. This shown the highest yield produced which is 90%. The curve start to decline when the time is increases to 50 minutes and even more at 60 minutes. Probably because lower amount of lye, more hydroxide will be evaporated at high temperature instead of surfactant formation.

4.4 Effect of Temperature on Bio Surfactant Yield

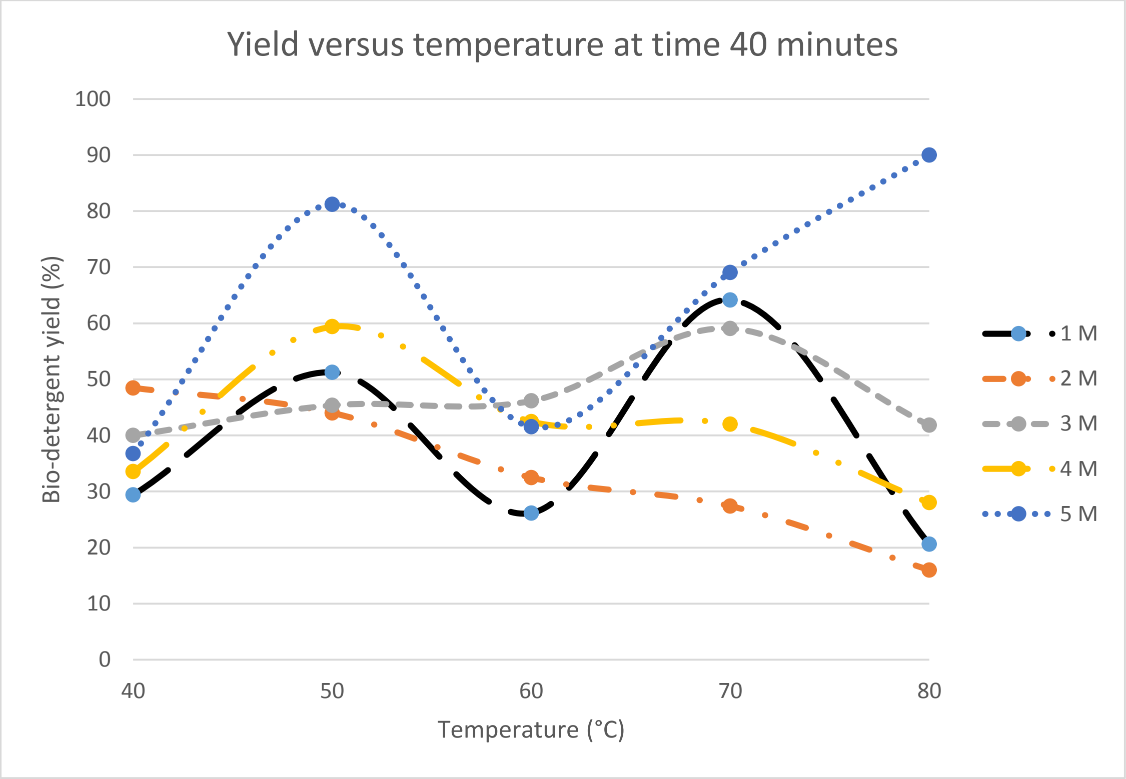


Figure 13: Yield versus temperature at time 40 minutes

From *Figure 13*, it shows the effect of time on the yield of bio surfactant at constant time. Each sample was run in a same process with a constant amount of oil at difference temperature and concentration. At 1 M, the curve is fluctuated. The yield at lowest temperature and time is relatively high and fluctuating through the constant parameter change. Started with increased slowly from 40 °C to 50 °C then dropped at 60 °C. Yield start to increased back at 70 °C and declined as increasing temperature.

At 2 M, yield started with the highest yield which is 48.46% and the curved started to drop as increasing the temperature. The lowest yield can be observed when the temperature at 80 °C. At 3 M, the bio surfactant yield increased as the temperature increased from 40 °C to 70 °C. But the yield suddenly plummet to 41.85% which is the lowest yield.

At 4 M, the yield shoot up to 59.38% when the temperature is further increased to 50 °C. Bio surfactant yield's decreased slowly from 50 °C to 60 °C .The curved was constant at 60 °C and 70 °C, then dropped slightly as increasing temperature. The highest yield was at 50 °C.

For concentration 5 M, the yield of bio surfactant are slow at the very beginning and rose at 50 °C. The curve shown that, yield drop at 60 °C and started to grow enormously until achieved the highest yield. The yield of bio surfactant formation was highest at high temperature and high concentration. According to Hauqe (2013), the temperature of surfactant or soap making need to be held at considerable temperature. Hence, excess hydroxide added will be able to hydrate the soap and evaporated to stabilize the pH.

4.5 Analysis of Bio Surfactant

Few analysis were run to determine whether the bio surfactant synthesis followed the criteria of standard detergent stated by ASTM. Three sample were chosen with the highest yield as the possible candidate as stated below.

Sample (Working)	1	2	3	ASTM D460 (Commercialized detergent)
Time (Min)	40	60	40	-
Concentration (M)	5	5	5	-
Temperature (°C)	50	40	80	-
pH	9	10	9	8
foam height (cm)	1	0.5	2.5	1.8
oil emulsification	D	D	D	D
hard water test	Layer	1.2	Layer	Layer
Percentage yield (%)	81.231	84.462	90	-

Table 2: Analysis of Sample and Blank

From Table 2, pH is one of the most common analyses in soil and water testing. Hydrogen (H^+) ions which are atom or groups of atoms with negative or positive charge that can control acidity levels. The level of pH is so important because aquatic organism need the pH of their water body to be within a certain range for optimal growth and survival. Mostly aquatic organisms prefer pH of 6.5 to 10 even though each organism has an ideal pH. Reproduction can be impacted by out of range pH, and organisms may even die if the pH gets too far from their optimal range. Furthermore, toxic will be released and compounds from sediments into the water where they may be taken up by aquatic animals or plants if the pH value was low (Addy *et al*, 2004). According to U.S.E.P.A considers lakes with pH less than 5 “acidified”. Aquatic organisms may be stressed in acidified lakes. Usually, the sea water pH criteria is 6.5 to 10 and for fresh water pH criteria of 6.5 to 9 or as what occurs naturally. The recommended pH for cleaning products range between 7 to 10. Nevertheless, the detergent with high pH have lower of fat contained. When contact with skin, the fat forming on the skin may causes hydration on skin (Jovian,2010). All sample can be accepted because nearly to the pH of commercialized detergent.

Water that contains calcium ions, Ca^{2+}, and magnesium ions, Mg^{2+} is said to be hard water. The greater the concentration of these minerals, the harder the water. Hard water doesn't clean as effectively as soft water. In hard water much of the surfactant added to the washer goes to soften the water instead of to clean the clothes. This means that more detergent must be used than in soft water. The levels relate to the concentration of dissolved minerals, which can be expressed in either grains of calcium and magnesium per gallon of water, parts per million, or milligrams per litre. Hard water interferes with almost every cleaning task from washing clothes, dish washing, bathing, personal care and many more that involved with cleaning. Example for clothes laundered in hard water, the fabrics of the clothes may feel harsh and scratchy. While for dish washing, the glass or dishes may be spotted when dry. Furthermore, hard water may cause a film on bathtubs, shower doors, sinks, faucets and many more. When hard water touch with skin surface, the skin become itchy and dry. Faucets and other fixtures can have permanent deposits on them and the chrome finish can be destroyed (Annelies *et al*,2005). Sample 1 and 3 can be accepted because form layer for hard water test.

Emulsion are either oil suspended in an aqueous water or water suspended in oil. In other word, emulsion means oil and water cannot be mixed together. Emulsifiers contain both hydrophilic head group and hydrophobic tail. Hydrophilic head is water loving or others name as polar while hydrophobic tail is oil loving or others name as non-polar. Emulsifiers will be attracted to both compounds. Surfactant are able to clean lies in its ability to disperse water insoluble molecules. When the mixture started to agitate, the oil will started to emulsify because of the charge on the surface and cause the mixture to disperse (Jovian,2010). All sample can be accepted because the oil dissolved in surfactant and same as commercialized surfactant criteria.

Foam will produced when surfactant diluted with water. Foam can be defined as the gasification process in a liquid. Foam is unstable and may disappear gradually but can stabilize with the aid of certain additives. To protect the fiber, stable foam is needed by reducing the mechanical action.

While for surfactant cases, high foaming is neither necessarily important nor desired. Furthermore, foam is often seen as a compulsory by the consumers because can give a good cleaning performance. Sample 1 and 3 are acceptably high when compared with commercialized detergent.

Sample 2 was rejected because of low foam height. Sample 1 and 3 have high foam height compared with commercialized detergent. But sample 1 was rejected because has low yield of bio surfactant. Sample 3 is chosen as bio surfactant due to highest yield.

5 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Waste cooking oil has been successfully synthesized as bio surfactant. It can be used as an alternative renewable bio surfactant feedstock. Furthermore, by recycling waste cooking oil, pollution can be avoided. From waste, it can generate money and at the same time it can save the world. The bio surfactant synthesis from waste cooking oil using alkali base was affected by process parameter. The highest bio surfactant yield (90%) was found by the concentration at 5 M, time of 40 minutes and temperature of 80 °C. The physiochemical properties of the synthesized bio detergent fulfilled the ASTM D460.

5.2 Recommendation

For recommendation, some ideas might be used to improve the result in the future. The recommendation that need to be considered are

- More study on the chemical used to make bio surfactant
- Using different raw material
- Develop new method and system

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APPENDICES

Appendix A1: Result Data At Concentration 5 M

Minute	20				
Temperature	40	50	60	70	80
Weight	8.96	7.25	8.39	7.498777	8.1
Yield	37.8462	11.5385	29.0769	15.3658	24.6154
pH	10	9	10	9	10
foam height	1	1.5	1	1.3	2
oil emulsification	D	D	D	L	D
hard water test	L	1.2	L	1	L

minute	30				
temperature	40	50	60	70	80
weight	7.9	8.791419	7.72	9.440377	7.61
yield	21.5385	35.2526	18.7692	45.23657	17.0769
pH	10	9	10	9	10
foam height	0.5	1	1.5	1.8	1.8
oil emulsification	L	D	D	D	D
hard water test	L	L	L	L	L

minute	40				
temperature	40	50	60	70	80
weight	8.89	8.142345	9.2	7.907171	12.35
yield	36.7692	25.26684	41.5385	21.64878	90.000
pH	10	9	10	9	9
foam height	1.4	1.7	2	2.3	2.5
oil emulsification	D	D	D	L	D
hard water test	L	0.6	L	0.2	L

minute	50				
temperature	40	50	60	70	80
weight	10.7	10.3719	9.54	9.5785	10.68
yield	64.6154	59.5677	46.7692	47.36154	64.3077
pH	9	10	10	9	10
foam height	1.5	1	0.6	1.3	1.5
oil emulsification	L	D	D	D	D
hard water test	L	1.5	L	1.8	L

minute	60				
temperature	40	50	60	70	80
weight	11.99	9.966097	10.47	8.863862	9.44
yield	84.4615	53.32457	61.0769	36.3671	45.2308
pH	10	9	10	9	9
foam height	0.5	1	0.3	1.3	2.3
oil emulsification	D	D	D	D	D
hard water test	1.2	L	L	0.6	L

Appendix A2 : Result Data At Temperature 80 °C

concentration	1				
minute	20	30	40	50	60
weight	7.9100	7.4903	7.8400	8.5382	7.7100
yield	21.6923	15.2357	20.6154	31.3568	18.6154
pH	10	9	10	9	10
foam height	1.5	1.9	1	1.3	1
oil emulsification	D	L	D	L	D
hard water test	L	0.5	L	1	L

concentration	2				
minute	20	30	40	50	60
weight	8.6500	7.2379	10.1400	9.2600	10.9200
yield	33.0769	11.3527	56.0000	42.4615	68.0000
pH	8	9	8	9	10
foam height	1	1.5	0.5	1.6	1
oil emulsification	D	D	D	D	D
hard water test	L	1	L	L	L

concentration	3				
minute	20	30	40	50	60
weight	8.0400	7.1062	9.2200	9.9603	9.2400
yield	23.6923	9.3265	41.8462	53.2356	42.1538
pH	10	9	10	9	9
foam height	1	1.8	0.8	1.6	1
oil emulsification	D	D	D	D	D
hard water test	L	0.8	L	L	L

concentration	4				
minute	20	30	40	50	60
weight	9.6300	9.9619	9.2200	10.5533	9.0200
yield	48.1538	53.2605	41.8462	62.3584	38.7692
pH	10	9	10	9	10
foam height	1.7	1.9	1	2.3	2
oil emulsification	D	D	D	L	D
hard water test	L	L	L	1	L

concentration	5				
minute	20	30	40	50	60
weight	8.1000	7.2500	12.3500	8.5200	9.4400
yield	24.6154	11.5385	90.0000	31.0769	45.2308
pH	10	10	9	10	9
foam height	2	1.8	2.5	1.5	2.3
oil emulsification	D	D	D	D	D
hard water test	L	L	L	L	L

Appendix A3 : Result Data At Time 40 Minutes

temperature	40				
concentration	1	2	3	4	5
weight	8.4100	9.6500	9.1000	8.6800	8.8900
yield	29.3846	48.4615	40.0000	33.5385	36.7692
pH	10	9	10	9	10
foam height	0.7	0.5	0.6	1	1.4
oil emulsification	L	D	D	L	D
hard water test	L	L	L	L	L

temperature	50				
concentration	1	2	3	4	5
weight	9.8300	9.3600	9.4500	10.3600	11.7800
yield	51.2308	44.0000	45.3846	59.3846	81.2308
pH	10	9	9	8	9
foam height	1.2	1	1.5	1.8	1
oil emulsification	L	D	D	L	D
hard water test	L	0.5	L	L	L

temperature	60				
concentration	1	2	3	4	5
weight	8.2000	8.6100	9.5000	9.2600	9.2000
yield	26.1538	32.4615	46.1538	42.4615	41.5385
pH	10	9	10	9	10
foam height	1	2	2.3	2.5	2
oil emulsification	D	D	D	D	D
hard water test	L	1.2	L	L	L

temperature	70				
concentration	1	2	3	4	5
weight	10.6700	8.2800	10.3400	9.2300	10.9900
yield	64.1538	27.3846	59.0769	42.0000	69.0769
pH	10	9	10	8	9
foam height	1	2.3	1.5	1	1.2
oil emulsification	L	D	D	L	D
hard water test	L	L	L	1.5	L

temperature	80				
concentration	1	2	3	4	5
weight	7.8400	7.5400	9.2200	8.3200	12.3500
yield	20.6154	16.0000	41.8462	28.0000	90.0000
pH	10	8	10	10	9
foam height	1	0.5	0.8	1	2.5
oil emulsification	D	D	D	D	D
hard water test	L	L	L	L	L

Appendix A4 : Graphics

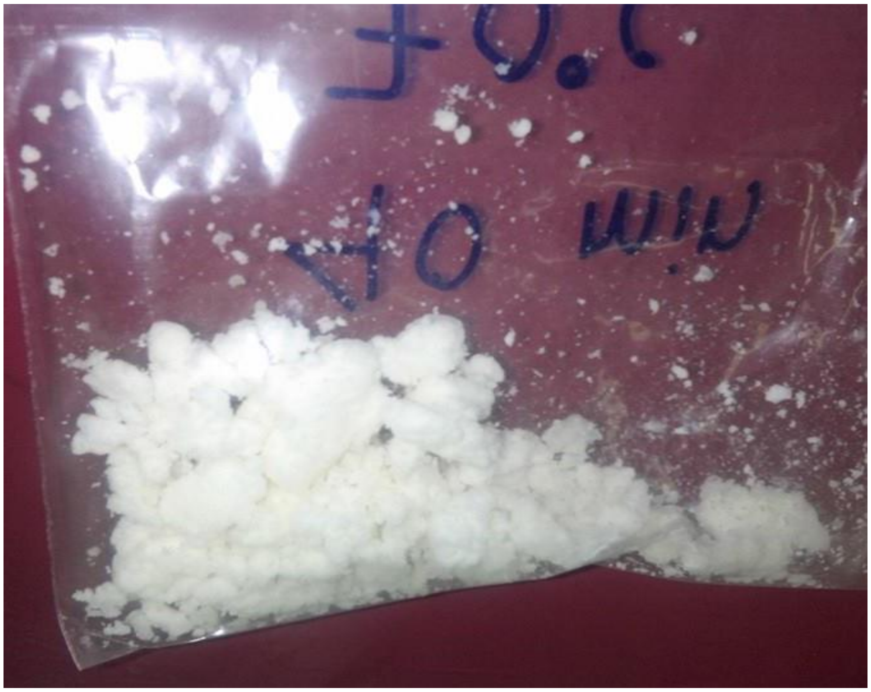

Figure 14: Bio surfactant from waste cooking oil

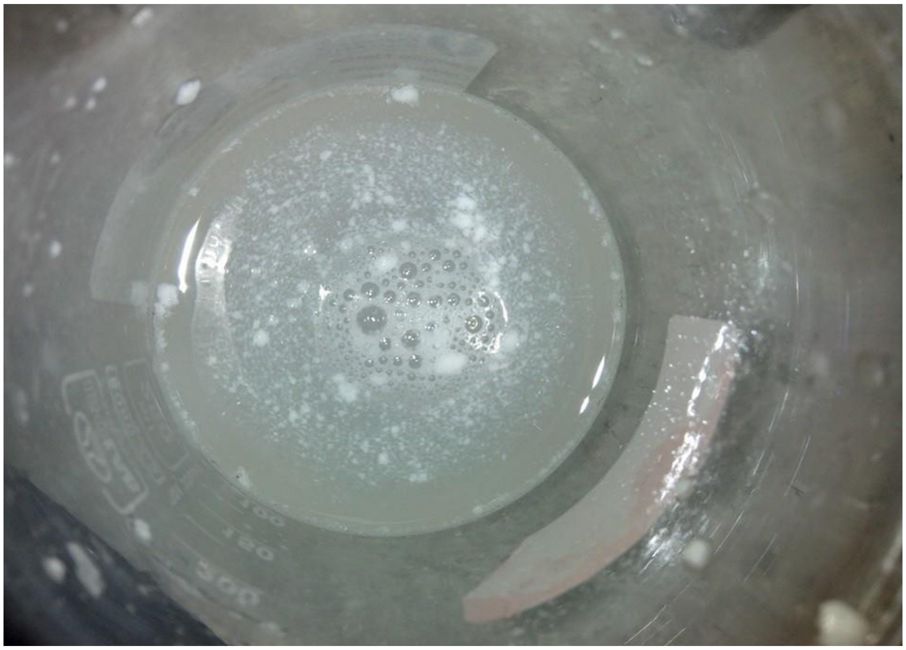

Figure 15: Oil emulsification of Bio Surfactant with Oil Analysis

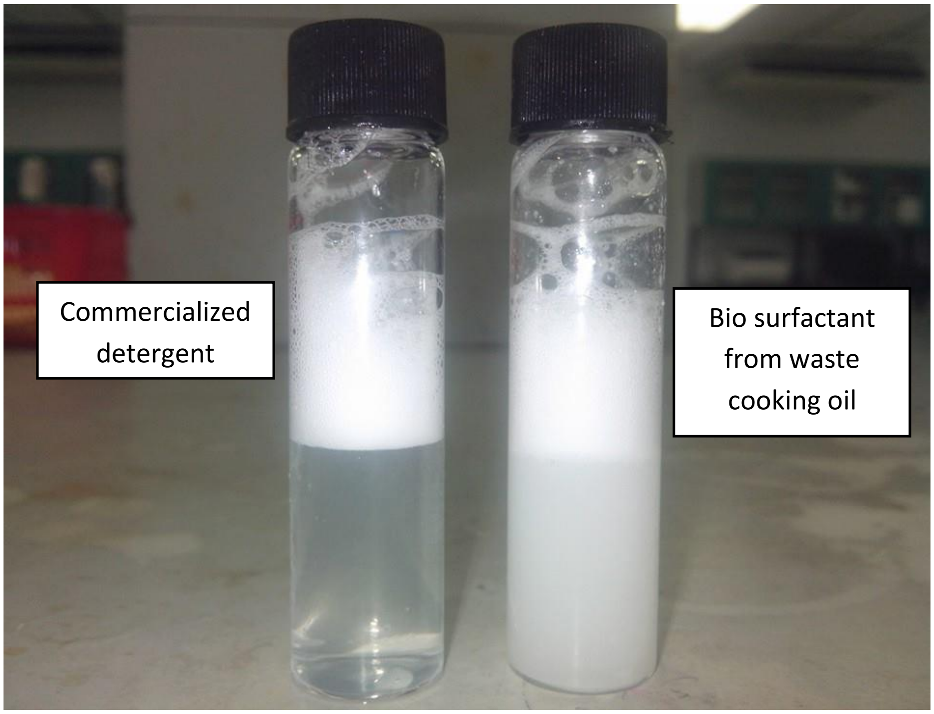

Figure 16: Foaming test

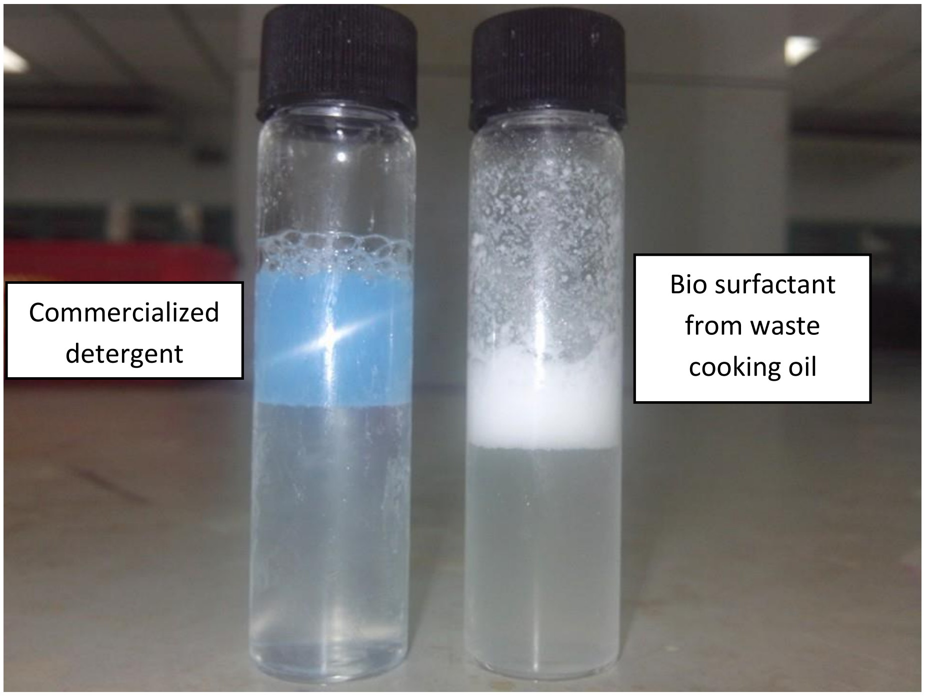

Figure 17: Comparison of hard water test between commercialized detergent and bio surfactant from waste cooking oil