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Physiochemical Properties of Biofilm from *Dioscorea hispida* Starch Blended with Glycerol Extracted from Recycling Cooking Oil

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Abstract. Glycerol is a by-product produced from biodiesel production through the transesterification process. The excessive amount of glycerol generated during this process may become an environmental problem since it cannot be disposed on the environment. One of the possible applications is its use in biofilm production as a plasticizer. This research aims to characterize the physicochemical properties of biofilm produced from *Dioscorea hispida* (Ubi Gadong) starch with a different formulation of glycerol extracted from recycling cooking oil. *Dioscorea hispida* starch has shown great potential as a major component in bioplastic production due to its faster degradation rate, while glycerol acted as a plasticizer. The contact angle, water absorption, water content, and degradation rate of biofilm were also studied. Biofilm with the highest ratio of glycerol content showed the highestwater absorption, which achieved 75.75%, and the biodegradable rate of biofilm was 97.99% on day 3. The lowest glycerol content in biofilm showed the lowest contact angle and completely biodegradationafter five days buried in the soil.

Introduction

Biofilm is commonly made from renewable materials, namely starch, cellulose, chitosan, and protein.Usually biofilm takes three to six months to fully degrade compared to synthetic plastic, which remains for 500 to 1000 years. Intentionally, biofilm development is to reduce the dependency on the petroleum-based, which is the main raw material in plastic production and increase carbon emissions. Presently, biofilm signified approximately one percent of the almost 300 million tons of plastic formed once a year. It is predicted that the overall biofilm fabrication volume will be around 2.44 million tons in 2022. Currently, 50% of the commercial biofilm was prepared from starch [1].

In petrochemical processing a significant amount of crude glycerol produced as by-product from biodiesel process by using recycled cooking oil. Using recycled cooking oil as a raw material is due to the sustainable alternative to raw vegetable oils and fats in biodiesel production, considering environmental and economic benefits [2]. For this reason, greater attention is given to the use of crude glycerol from biodiesel production to defray the cost of biodiesel production and to encourage large-scale biodiesel industrialization. However, this crude glycerol needs to undergo the purification process to be used in various application of high value-added product. Glycerol is commonly used as a plasticizer to produce starch-based biodegradable film. Starch and glycerol melt and flow at temperatures between 90 °C and 180 °C. Plasticizers could create greater flexibilityin the polymer structure by reducing the intermolecular forces and the materials glass transition temperature, which increases the mobility of the polymer chains in the starch plastic [3].

Dioscorea hispida or locally known in Malaysia as ‘ubi gadong’ is a starchy tuber plant which belongs to the genus *Dioscorea* within *Dioscoreaceae* family. It contain a significant amount of starch content. Starch is known as one of the most adaptable macromolecules and also a second largest natural biopolymer after cellulose. It is hydrophilic in nature, abundant, sustainable and fully biodegradable carbohydrate polymer that need low preparation costs. Basically, starch consists of two

major components which are namely amylose and amylopectin. Amylose is in a linear chain and amorphous which connected together by α-(1,4) glycosidic-linked bond while amylopectin exist as branched structure with an extra bond of α-(1,6) glycosidic - linked that mostly present as crystalline forms. Starch has been used primarily in food products for a long time, but today it also functions in various industries such as paper, textiles, adhesives, drinks, confectionery, plastics, including pharmaceutical application [4]. In this study, starch was extracted from *Dioscorea hispida* tuber and used in the production of biofilm. Purified glycerol that obtained from recycle cooking oil was blended in the formulation of biofilm. Physiochemical properties of biofilm produced from this formulation was investigated.

Materials and Methods

Extraction starch from *Dioscorea hispida* tuber. *Dioscorea hispida* tuber was collected from Tanah Merah, Kelantan. The tuber was cleaned and peeled before cutting into small cubes shape. The small cubes of *Dioscorea hispida* tuber were mixed with a small amount of water until it was blended into an even paste. The paste was filtered using cloth filter and squeezed it for several times until the residue of the tubers partially dried. After that, the solution was left for 24 hours in the chiller. The solution was separated where the starch was on the bottom layer of the beaker while the upper layer was water. The upper layer was discarded and starch was collected. The starch solution was poured onto aluminum foil and leave it in the oven to dry for 24 hours at 50 ºC. The dried starch was blended with a hand blenderuntil it produced a fine powder (Fig.1). The starch powder was kept in an air-tight bag to maintain the relative humidity of the sample moisture and avoid contamination.

Fig. 1. Starch extract from *Dioscorea hispida* tuber

Production of glycerol from recycled cooking oil. 100 mL methanol was stirrer stirred with temperature of 45 °C and 3.5 g sodium hydroxide pellets was slowly added. The methanol and sodium hydroxide were mixed until the solution was completely dissolved about 15 to 20 minutes. This reaction produced sodium methoxide. At the same time, 500 mL waste cooking oil was heated on a hot plate at 50 –55 °C. Then the mixture was mixed into heated waste cooking oil. This mixture was blended at a speed of 1000 rpm for 20 minutes to avoid the formation of a layer. The mixture was poured into a 1 L separating funnel. The liquid was started to separate out into two layers. The mixture was left for 24 hours. After 24 hours, the mixture was observed where the bottom layer was crude glycerol and the top layer was biodiesel. The crude glycerol was collected from the separating funnel.

Production of Biofilm from *Dioscorea hispida* starch and glycerol from recycle cooking oil. Table 1 below shown that biofilm were produced from *Dioscorea hispida* starch with different ratio of glycerol and distilled water. The biofilm, as a control was produced from commercial starch, commercial glycerol and distilled water. Based on Table 1, six biofilms were prepared with the weight of starch used was constant while the volume of glycerol and distilled water was different depended on the ratio of the formulation from each mixture of 100 mL.

Table 1. The different ratio of formulation biofilm.

Biofilm	Starch (g)	Glycerol (mL)	Distilled Water (mL)
Control	5	1	94
1	5	1	94
2	5	2	93
3	5	3	92
4	5	4	91
5	5	5	90

Starch and glycerol were diluted with distilled water in 500 mL beaker. The mixture was slowly heat and stirrer at 2000 rpm on the hotplate until it reached 70 ºC for 2 hours. The heat button was turned off and leave for 24 hours. The solution was stirred at 3000 rpm to avoid the hardening of mixture. After 24 hours, 10 mL of solution was poured on a disposable petri dish and dried in the oven at 50 ºC for 3 hours. The biofilm were obtained and kept for further analysis.

Analysis the properties of biofilm. In this study, a contact angle test was carried out to determine the hydrophobicity of a solid surface. This was done by examining its wettability. One drop of distilled water was dropped on each bioplastic sample. However, the sample was placed between the light and the camera at a close angle. For the contact angle calculation, this required a flat baseline to be calculated. The contact angle point, according to the wettability of the solid material, will range from 0 to 180 °. The 0° reveals the materials strongly hydrophilic nature and the 180° hydrophobic material. Water absorption test was carried out to determine the ability of bioplastics to absorb water where was identified by standard ASTM D 570. The dried biofilm was cut into 2 cm x 2 cm and the weight of dried bioplastic samples was recorded. The biofilm samples were soaked into each beakers contained 200 mL of water for 24 hours. Then, the bioplastic samples were wipe dried with filter paper to absorb excessive water. The final weight of biofilms samples were achieved to obtain water absorption data. The water absorption percentage was calculated according to the Equation 1;

$$\text{Water absorption (\%)} = \frac{W_{final} - W_{initial}}{W_{initial}} \times 100 \qquad \text{Eq. 1}$$

Biodegradability of the biofilm samples were determined by soil burial test. The bioplastic samples were cut into 2 cm x 2 cm and buried into ground at depth of 6 cm. The location of burial test was at backyard of building block A. The initial weight of bioplastic samples were recorded and final weight of biofilm samples were recorded from day 1 until day 7. The biodegradability test was measured by Equation 2:

$$\text{Biodegradability (\%)} = \frac{W_{initial} - W_{final}}{W_{initial}} \times 100 \qquad \text{Eq. 2}$$

Results and Discussion

Biofilm Production from *Dioscorea hispida* starch. Biofilm from *Dioscorea hispida* starch havebeen successfully produced. The physical observation of surface morphology properties of biofilm was smooth, transparent and soft yellowish in colour as shown in the Fig.2.

Fig. 2. Biofilm production.

Contact angle. In this work, the contact angle were obtained where the droplet on biofilm (control) showed the incomplete wetting while the biofilm sample was shrink immediately as in Fig. 3.

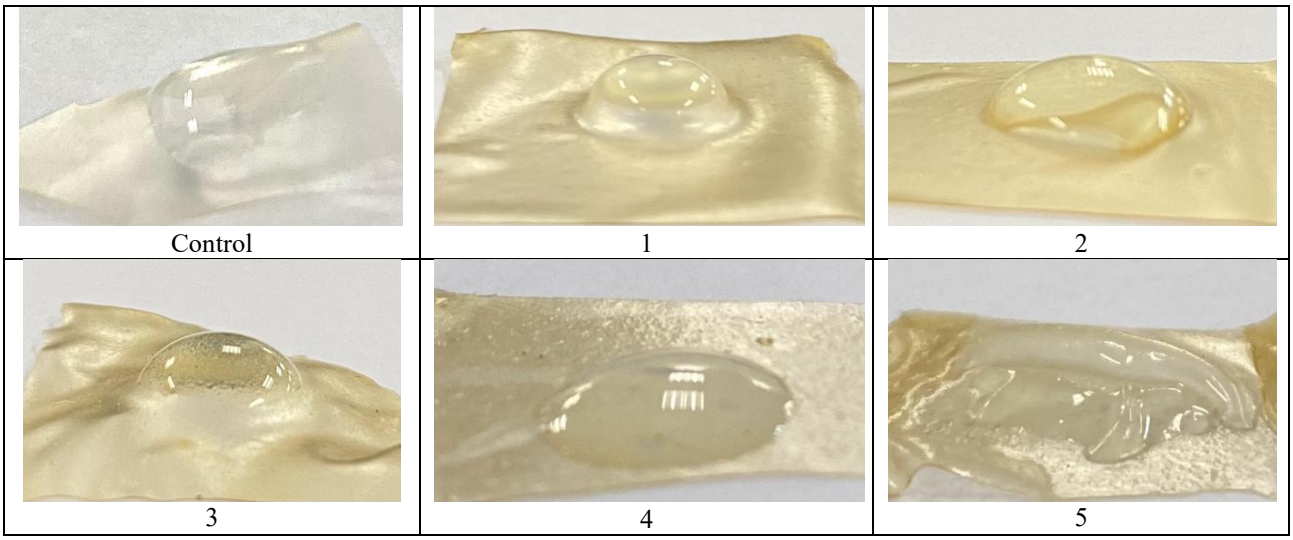

Fig. 3. Contact angle of biofilms with different ratio of glycerol and starch.

The biofilm (control) showed that the droplet of water was unable to absorb the water. Biofilm 1 and 2 were incomplete wetting, while biofilm 3 and 4 were good wetting. The biofilm 5 was the only one that shown spreading the droplet of water on the surface. Biofilm 5 had the highest volume of the glycerolshowed a low contact angle and treated to be hydrophilic due to the properties of glycerol which was hygroscopic. The low contact angle indicated that it has high surface energy and high interfacial tension. Therefore, biofilm 1 and control with the lowest volume of glycerol have a higher contact angle and was considered hydrophobic. This is because the surface energy weaker than the surface tension of the liquid which means the droplet of water can maintain their shape on the surface [5].

Water absorption. Water absorption test was essential in order to determine the capacity of plastic or a polymer to absorb water from its surroundings was the absorption of water. Table 2 shows the percentage of the water absorption on a different ratio of the biofilm. The biofilm 5 with 5 mL glycerol content showed the highest percentage of water absorption which was 75.75 % while biofilm (control) had the lowest percentage of water absorption which was 20.41 %.

Table 2. Water absorption of biofilm samples with different ratio of glycerol and starch.

Biofilm samples	Initial weight (g)	Final weight (g)	Water Absorption (%)
Control	0.1303	0.1569	20.41
1	0.1245	0.1689	35.66
2	0.1430	0.2124	48.53
3	0.1485	0.2295	54.55
4	0.1528	0.2541	66.30
5	0.1489	0.2617	75.75

The reduction in absorbed water then leads to a decrease in the amount of hydrolyzed biofilm polymer chains. According to the result, the higher content of glycerol used in production of biofilm affecting the water absorption on the bioplastic due to the polar properties of glycerol which was soluble in water and natural hygroscopic. This was due to the tendency for the glycerol to absorb moisture from the surrounding was higher. Besides, the presences of three hydroxyl groups in a glycerol molecule are also influencing the tendency for water absorption of glycerol-plasticized composites and solubility of water. Therefore, the higher content of glycerol in bioplastic resulted in poor properties of plasticizer. Basiak et. al., (2018) stated that plasticizers are usually more

hygroscopic than starch in starch films. Indeed, Muscat et. al., (2012) indicated that with polyol content, the amount of water absorbed by starch-based plastic increases. Therefore, the variation in the ability of starch-based plastic to adsorb water (when the quantity of starch constant) depends mainly on the concentration of the plasticizers used.

Biodegradability of biofilm. Table 3 indicate the biodegradability of biofilm with different ratio ofglycerol and starch after 7 days. As result shown, the longer the burial period, the greater biofilm weight loss, which suggests the greater biofilm degradation was developed. The rate of biodegradability was increased every day to 99.69% on day 4.

Table 4. Biodegradability of biofilms until days 7

Bioplastic	Biodegradation Rate Of Bioplastic (%)						
	1 day	2 days	3 days	4 days	5 days	6 days	7 days
Control	28.20	53.08	84.03	99.38	-	-	-
1	32.63	56.80	86.86	99.69	-	-	-
2	35.31	59.09	88.55	-	-	-	-
3	38.47	63.45	92.54	-	-	-	-
4	40.98	66.43	95.17	-	-	-	-
5	43.06	68.85	97.99	-	-	-	-

According to ASTM standard, decomposing time for starch-based plastic to completely degraded was 12 days. The biofilm (control)had the lowest percentage of biodegradability among the other biofilm due to the composition itself. Commercial tarch has the longest shelf life as it was for commercial purposes. A reduction in molecular weight or shortening of the long chain was caused by chemical degradation reactions inlinear polymers. Starch has acetal bonds that can be degraded very quickly. Due to the apparent biofilm composition, the scale of this weight reduction was a natural substance that was digested rapidly bymicroorganisms. A natural polymer having a hydroxyl group (-OH) was the primary factor of a polymer that could be spontaneously degraded, so microorganisms easily degraded this group.Temperature, oxygen, humidity, and microbial factors of biodegradable polymers significantly control the effects and mechanism of biodegradation of film materials. Increasing the amount glycerol decreased the residual biofilm mass and improved biodegradability due to the hydroxyl group in starch would trigger the polymer's hydrolysis reaction after the material absorbed water, whereas glycerol, a hydrophilic plasticizer, effectively reduced the polymer chain's internal hydrogen bonds [8].

Summary

In this research, glycerol that produced from biodiesel process was able to replace commercial glycerol as it sustainable and low processing cost than commercial. The use of different glycerol ratios in biofilm affected the mechanical, physical, hydrophobicity and biodegradability properties of starch-based plastic. The biodegradability of biofilm made from *Dioscorea hispida* starch was affected by the amount of glycerol content used as a plasticizer. The greater the amount of glycerol, the higher the biodegradation rate, water absorption rate and smaller of contact angle. These results indicated that the formulation of glycerol from recycling cooking oil and *Dioscorea hispida* starch would be a good base material for the production of biofilm and its application as food packaging at the same time it increase the awareness toward recycling the waste cooking oil into high value added product.

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