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Preliminary study of biodegradability of starch-based bioplastics using ASTM G21-70, dip-hanging, and Soil Burial Test methods

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Abstract. Biodegradability testing methods being used nowadays have many disadvantages; they are time-consuming, inefficient medium used, and too much sample needed to do the test. This work aimed to study the biodegradability of starch-based bioplastics by modified ASTM G21-70 method using Salt Agar (SA) medium, dip-hanging method using sterile water, and Soil Burial Test (SBT) method. Bioplastics were prepared by mixing cassava starch and glycerol with a ratio of 3:1 (% w/w) through a series of processes: (1) blending of starch and glycerol for 3 min, (2) extruding of the starch-glycerol mixture by using a single screw extruder at 80-130°C, and (3) compression molding at temperature and pressure of 150°C and 50 kgf/cm², respectively. *Aspergillus niger* was used as bioplastic-degrading fungi for the modified ASTM G21-70 and dip-hanging methods, while compost-soil was used as a source of bioplastic-degrading microbes in SBT method. Bioplastics of 2x2 cm in size were applied to the tests for 10 days. The growth of fungi on the surface of bioplastics was observed visually at two days intervals. *A. niger* grew well on the surface of bioplastic sample in modified ASTM G21-70 method, indicated that the bioplastic could be degraded by the fungi. On the other hand, the growth of *A. niger* was poor in the dip-hanging method, even though weight loss of 11.5% occurred. Physical properties changing were indicated in the SBT method. On the 10th day, cracks were observed on the surface of the bioplastic sample, the color of the sample became darker even the bioplastic became fragile, and the weight loss reached 29.89%.

1. Introduction

Conventional plastic used by people around the world is a polymer from petroleum or natural gases. Most conventional plastic ingredients are synthetic polymers that are difficult to decompose naturally in less than 50 years. According to this character of conventional plastic, plastic waste will stack in the landfill and caused environmental damage such as inhibit water flow, water pollution, etc [1].

One of the approaches to reduce the negative impact of plastic waste is by using bioplastic, which has similar uses as conventional plastic but will be degraded by the activity of microorganisms after disposal to the environment [2]. Bioplastics commonly are prepared from starch as the main component due to the nature of starch, which is easily degraded into environmentally friendly compounds. Moreover, starch has several advantages such as the ease of processing and affordable, owing to a large number of starch-producing plants in Indonesia, such as cassava, corn, rice, potato, and peanut [3].

One of the sources of starch is cassava that can be processed into tapioca flour, the pure starch obtained from the extraction of cassava mills [4]. Amylose content in tapioca flour is in the range of



12.28% to 27.38% and amylopectin content is in the range of 72.61% to 87.71%. Amylose content affects the mechanical properties of bioplastics [5], while amylopectin content would provide the optimum stickiness of starch [4]. In bioplastic processing, the addition of plasticizers is desirable to improve the mechanical properties of bioplastic, particularly the elasticity. Glycerol is widely used as a plasticizer in fabricating bioplastic [3].

Biodegradation is a chemical process that occurs due to the presence of microorganisms in the environment that depend on ambient conditions (temperature, light, oxygen, humidity) and chemical structure. During the biodegradation process, the materials are converted into natural compounds such as water, CO₂, and compost [6]. The occurrence of degradation is characterized by the increase of per cent weight loss or the decrease of residual weight of bioplastic [7].

In this study, we performed three biodegradability test methods, which are modified ASTM G21-70 method using SA medium and without medium by using *A. niger* as test fungi, and Soil Burial Test (SBT). ASTM G21-70 is the standard method to identify the resistance of plastic to fungi growth. This method is adopted by the Indonesian National Standard (SNI) to check the biodegradability of plastic qualitatively. The dip-hanging method is a modified ASTM G21-70 method, but it needs no media. SBT method is a standard method to identify the degradation of bioplastic quantitatively. Biodegradability of starch-based bioplastic was evaluated by observing the physical changes such as color changes and weight loss of bioplastic for 10 days.

2. Materials and methods

2.1. Materials

Cassava Starch was obtained from Budi Starch & Sweetener Tbk (Lampung, Indonesia). Glycerol was purchased from Wilmar Nabati (Indonesia). Salt Agar (SA) media (KH₂PO₄, MgSO₄·7H₂O, NH₄NO₃, NaCl, FeSO₄·7H₂O, ZnSO₄·7H₂O, MnSO₄·7H₂O, and K₂HPO₄) were purchased from Merck (Darmstadt, Germany). *A. niger* was obtained from the Research Centre for Biotechnology LIPI (Cibinong, Indonesia). Compost soil was obtained from Setia Tani Group (Tangerang, Indonesia). All other chemicals were analytical grade and were purchased from Merck (Darmstadt, Germany).

2.2. Methods

2.2.1. Bioplastic preparation. Bioplastic was prepared by mixing cassava starch and glycerol with w/w ratio of 3:1 [8]. Starch and glycerol were mixed by using a single screw extruder at 80–130°C for 3 min. The mixture was then pelletized by using a compression molding at 150°C under a load of 50 kgf/cm².

2.2.2. Modified ASTM G21-70. Qualitative biodegradability tests were carried out by using a modified ASTM G21-70 method [9]. This method commonly uses some fungi and bacteria, but in this study, we used only *A. niger* as the decomposing agent because *A. niger* was easy to be identified as contaminant fungi widely spread in the air. *A. niger* was homogenized with 10 mL sterile aquadest using a wire loop to obtain spore suspension as the stock culture. Into 9 mL of sterile aquadest, 1 mL stock culture was added and the mixture was mixed by vortex to get a 10⁻¹ culture. Into 9 mL of sterile aquadest, 1 mL of 10⁻¹ culture was added and the mixture was mixed by vortex to get a 10⁻² culture. An amount of 100 µL of 10⁻² culture was added into a sterile petri dish; this procedure was performed in duplicate. Each dish was added with 20 mL of SA media (44°C-47°C). Petri dish was then stirred to make the mixture homogenous and the mixture was left for about 2 h until it became solid. After 2 h, samples of bioplastic (3x3 cm), synthetic plastic (negative control) and filter paper (positive control) were each placed onto solid SA media surface. One-hundred µl of 10⁻² culture was spread equally onto bioplastic sample and both controls. Bioplastic samples and controls were incubated at 30°C for 10 days.

Table 1. Biodegradability rank of bioplastics.

Score	The growth of fungi on the surface of bioplastic samples (%)
0	0
1	10
2	10-30
3	30-60
4	60-100

The growth of fungi in bioplastic samples was evaluated by biodegradability score as presented in Table 1. Score 0 indicated there is no fungi growth activity, which means the bioplastic is difficult to degrade. Score 4 showed the highest fungal growth, which means the biodegradability level is high.

The weight loss of the sample was measured by weighing samples before inoculation as initial sample weight (W_0) and after 10 days of inoculation process (W_f). Per cent weight loss was calculated with the following equations:

$$\% \text{ Weight loss} = \frac{W_0 - W_f}{W_0} \times 100\% \quad (1)$$

2.2.3. Dip-hanging method. *A. niger* isolate was added with 10 mL sterile aquadest (10^0). Bioplastic samples (3x3 cm) were dipped on *A. niger* mold in sterile aquadest (10^0) for 10 s. Each sample was then hung in a jar containing sterile aquadest for 10 days. Bioplastic samples were then incubated at 30°C for 10 days. The growth of *A. niger* mold was observed and the weight loss of bioplastics after 10 days was calculated using equation (1). Moisture absorption of the samples was then measured by using a moisture balance apparatus [7].

2.2.4. Soil Burial Test. Bioplastic samples (1x1 cm) were buried in compost soil at 7.5 cm depth, then incubated at room temperature for 10 days with sampling time every two days. The buried samples were then cleaned from the soil and weighed. The weight loss of the bioplastics was calculated using equation (1) [10].

3. Results and discussion

3.1. Biodegradability test of bioplastic using modified ASTM G21-70

The results of the biodegradability test with modified ASTM G21-70 method on SA media are shown in Figure 1. On day 0, there was no *A. niger* growth on SA media nor the bioplastic sample. *A. niger* began to grow on the 2nd day; they grow on bioplastic surface but not on the SA media surface. On day 4, the *A. niger* began covering the surface of bioplastic sample, but still not on SA media. *A. niger* began to grow to cover the bioplastic surface on the 6th day and on that day, the black colour of *A. niger* spores can be seen on bioplastic sample. On day 8, the density of *A. niger* biomass on both bioplastic and SA media increased from that of the 6th day. Almost all of the sample surface was covered on day 10. However, the growth of *A. niger* on SA media, which was a minimum media, was still poor compared to the growth on bioplastic samples. *A. niger* covered 80% of the surface of bioplastic sample on day 10. The weight loss on day 10 was 20.18%. Cassava starch, the main material of bioplastic samples, is rich in carbohydrate and contains an efficient amount of protein as a nitrogen source for the growth of the fungi [11]. Moreover, SA media provides only minerals and no additional carbon source. Therefore, much more fertile fungal growth was observed on the surface of the bioplastic sample than on SA media. The same growth was shown by filter paper, the positive control, while no growth at all was observed on negative control. The synthetic plastic had no nutrition for the growth of *A. niger*, in other words, synthetic plastic had high resistance properties against *A. niger*.

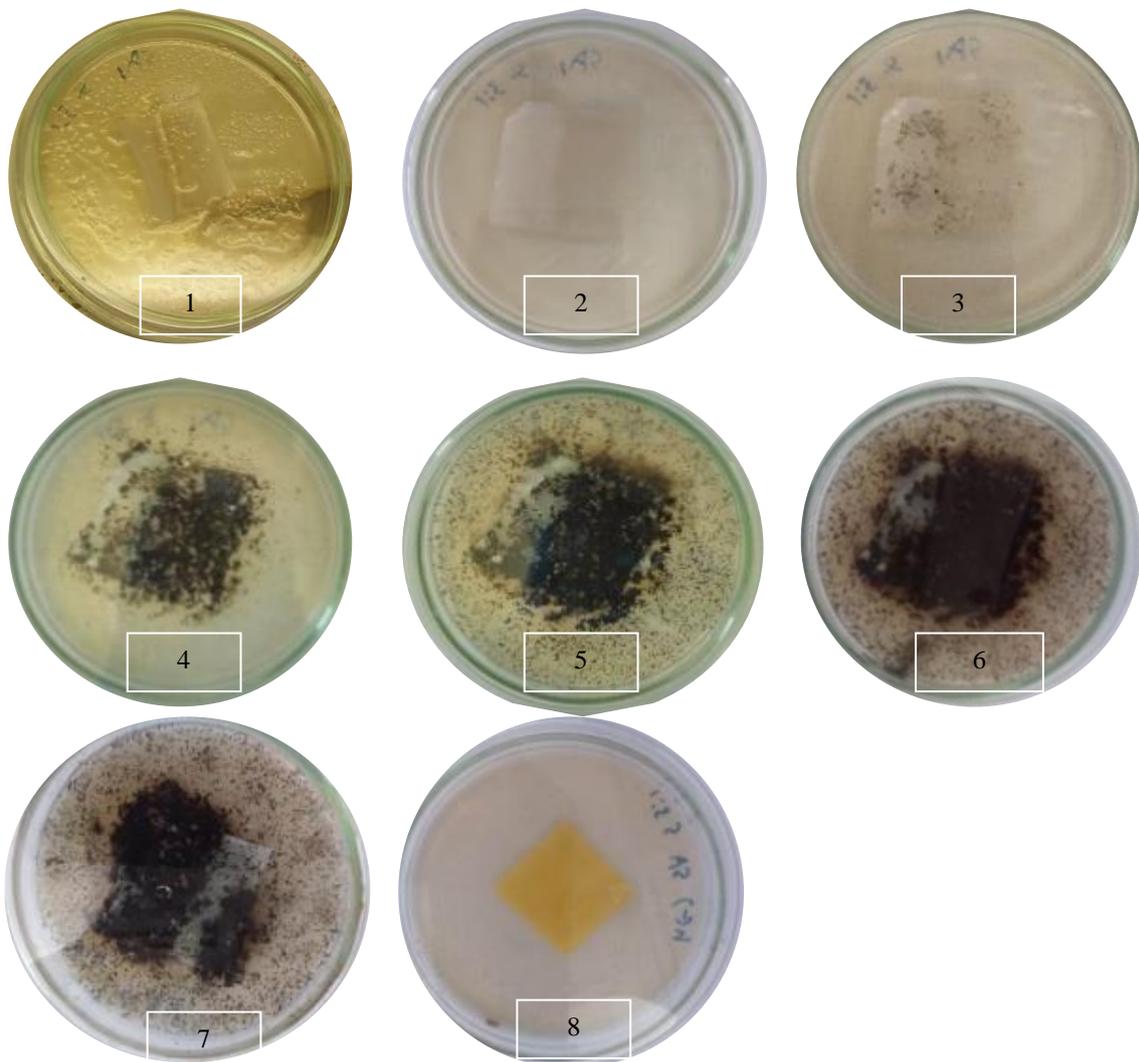


Figure 1. Biodegradability test of starch bioplastic on SA media at different incubation time. (1) No growth at day 0, (2) Mycelia began to grow on day 2, (3) Spore began to grow on day 4, (4) Spore began covering bioplastic surface on day 6, (5) Spore began covering SA media surface on day 8, (6) 80% of bioplastic surface were covered on day 10, (7) Positive control (filter paper) on day 10, (8) Negative control (synthetic plastic) on day 10.

3.2. Biodegradability test of bioplastic using the dip-hanging method

The results of biodegradability test with dip-hanging method are shown in Figure 2. For dip-hanging method, the carbon source for the growth of *A. niger* only came from bioplastic starch samples. It can be seen that the growth was less than the results of a modified ASTM G21-70 method.

The level of *A. niger* growth in the sample was interpreted in the form of score 0 to 4 as shown in Table 1. For dip-hanging method, the growth of *A. niger* was not optimal due to the lack of nutrition available, because the nutrition came only from the bioplastic itself so that the degradation level could not be scored. This was different from the modified ASTM G21-70 method that gave the *A. niger* minerals and an additional carbon source from SA media. Growth media for fungi must contain macronutrients in the form of carbon (C), nitrogen (N), phosphate (P), potassium (K), magnesium (Mg) and micronutrients in the form of iron (Fe), zinc (Zn) and manganese (Mn). Macronutrients and micronutrients elements affect cell multiplication and growth [12].



Figure 2. Biodegradability test of starch bioplastic by dip-hanging method. (1) No growth on day 0, (2) Mycelia began to grow on day 2, (3) The number of mycelia were increasing on day 4, (4) Spores began to grow on day 6, (5) The number of spores were increasing on day 8, (6) No significant change on day 10.

The factors that influenced the degradation process are the amount of fiber and environmental conditions, including water activity that related to humidity. Bioplastic samples can be degraded properly by *A. niger*, which is one of the soil microorganisms that degrade materials and break down complex elements into simple ones but will take a long time. To degrade plastic, microbes must be able to contaminate the surface through electroscopic counting and microbes must be able to use components in or on the plastic layer of nutrients [13]. The weight loss on day 10 was 11.46%. By hanging the samples, some of the water that was in contact with the sample would evaporate easily, even though the samples were hung in the chamber that contained sterile water. Insufficient water content of samples might cause poor growth of the fungi.

3.3. Biodegradability test of bioplastic using Soil Burial Test

The results of the biodegradability test using SBT method are shown in Figure 3 and 4. It can be seen that the longer the burial time, the higher the weight loss of bioplastic that means the higher the degradation of bioplastic occurred. The number of weight loss was increasing each day until 29.89% on day 10. According to ASTM standard, decomposing time for plastic film PLA from Japan and PCL from England is 60 days. Chemical degradation reactions in linear polymers cause a decrease in molecular weight or length chain shortening. Starch has acetal bonds which are very easy to degrade. The magnitude of this mass reduction was due to the bioplastic composition was a natural material that is easily digested by microbes. The main factor of a polymer that can be degraded naturally was a natural polymer containing a hydroxyl group (-OH), accordingly this group was easily degraded microbially [3].

The rate and mechanism of biodegradation of plastic materials are strongly influenced by temperature, oxygen, humidity and microbial conditions of polymeric materials. Increasing the amount of starch and glycerol made the bioplastic residual mass decreased and biodegradability increased due to the hydroxyl group in starch would initiate the hydrolysis reaction of the polymer after absorbing water from the media, while glycerol which was a hydrophilic plasticizer effectively reduced internal hydrogen bonds in the polymer chain [13].

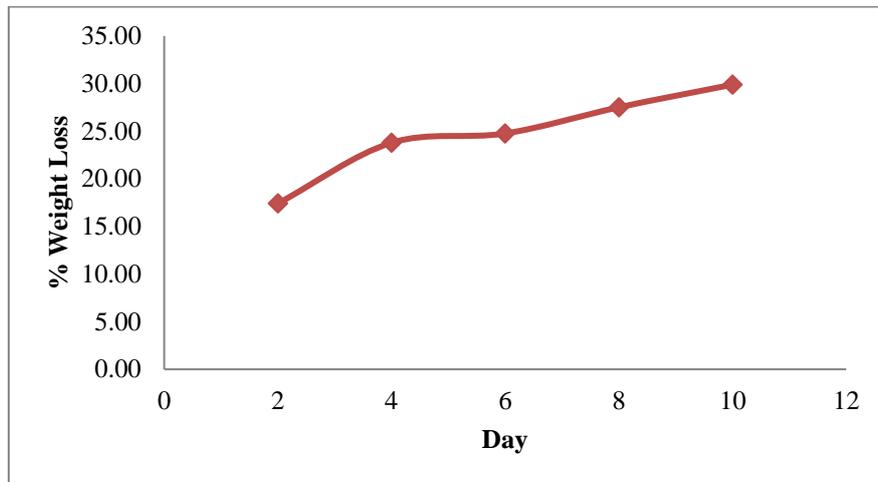


Figure 3. Weight loss of starch bioplastic after 10-days test with SBT method

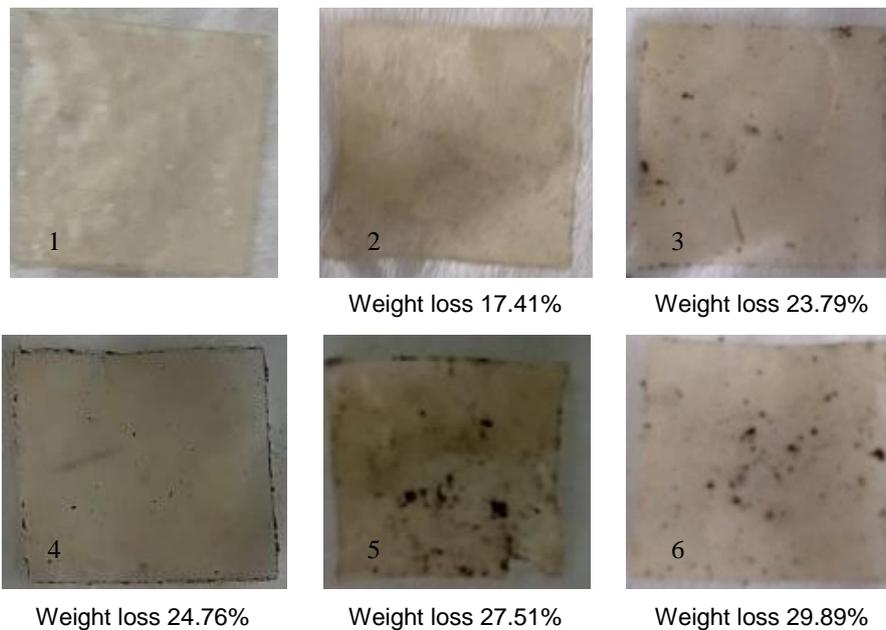


Figure 4. Soil burial Test. (1) Day 0, (2) Increasing size of bioplastic on day 2, (3) More soil water and the soil itself are entered the bioplastic pores on day 4, (4) Wider cracking area on bioplastic sample on day 6, (5) Some parts of bioplastic were destroyed on day 8, (6) Wider parts of the bioplastic were destroyed on day 10.

As shown in Figure 4, start from day-4, the bioplastic sample began to change physically where cracks were observed on the surface of the sample. Then on the 8th and 10th days, the form of the sample became fragile. Compared to another study [14], after burial for 90 days, the sample's dimension decreased and the sample became hard and fragile. Soil degradation greatly influenced changes in macrostructure. It is possible that the soil had more nutrients and synergistic works occurred among the activities of several microbes (fungi and bacteria) and caused the damage of macrostructure of bioplastic samples.

4. Conclusion

In the modified ASTM G21-70 method, *Aspergillus niger* grew better with 80% growth of mycelia covered the bioplastic surface, while in the dip-hanging method the growth could not be scored. Compost soil caused macrostructural changes of bioplastics in SBT method, with a weight loss of around 30%. ASTM G21-70 is an appropriate method to study the degradability of bioplastic qualitatively, and SBT is a suitable quantitative method to study biodegradability of bioplastics.

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