

Effect of *Tithonia diversifolia* extract on the biodegradability of the bioplastics in plantation soil

Pengaruh ekstrak Tithonia diversifolia terhadap biodegradabilitas bioplastik di tanah perkebunan

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Abstrak

Pengaruh ekstrak Tithonia diversifolia terhadap biodegradabilitas bioplastik dievaluasi dengan menggunakan tanah perkebunan sebagai inokulum alami. Bioplastik yang digunakan adalah komposit selulosa dari kulit buah kakao, pati dan diperkaya dengan ekstrak tithonia. Uji biodegradasi dilakukan di dalam botol selama 60 hari. Karbon dioksida yang dihasilkan dari uji biodegradasi diserap oleh larutan natrium hidroksida 0,1 N. Karbon dioksida dititrasi dengan HCl 0,1 N dan menggunakan fenolftalein diikuti dengan metil jingga sebagai indikator. Karbon dioksida terdeteksi pada sampel bioplastik namun tidak terdeteksi pada sampel plastik konvensional. Bioplastik yang diperkaya dengan ekstrak tithonia menghasilkan tingkat biodegradasi yang lebih tinggi dari pada bioplastik tanpa ekstrak tithonia. Tingkat biodegradasi sampel bioplastik di tanah perkebunan adalah 0,068 mg CO₂/hari dan 0,188 mg CO₂/hari masing-masing untuk bioplastik tanpa dan dengan ekstrak tithonia. Biodegradasi sampel bioplastik selama 45 hari adalah 12,44% dan 28,07% berturut-turut untuk bioplastik tanpa dan dengan ekstrak tithonia. Biodegradasi keseluruhan bioplastik diperkirakan membutuhkan waktu 244 hari dan 200 hari masing-masing untuk bioplastik tanpa dan dengan ekstrak tithonia.

[Keywords: *Tithonia diversifolia*, biodegradabilitas, bioplastik, tanah perkebunan].

Abstract

Effect of *Tithonia diversifolia* extract on biodegradability of the bioplastic was evaluated using plantation soil as natural inoculum. The bioplastic was a composite of cellulose from cacao pod husk, starch and enriched by tithonia extract. Biodegradation test was conducted in the glass jar for 60 days. The carbon dioxide generated from the biodegradation test titrated by 0.1 N sodium hydroxide solutions. The carbon dioxide was

measured with 0.1 N HCl and using phenolphthalein followed by methyl orange as indicator. Carbon dioxide was detected in the bioplastic samples but not detected in the conventional plastic sample during the test. Biodegradation of the bioplastic enriched by tithonia extract was higher than that of the bioplastic without tithonia extract. Biodegradation rate of the bioplastic samples in plantation soil were 0.068 mg CO₂/day and 0.178 mg CO₂/day for the bioplastic without and with tithonia extract, respectively. Biodegradation of the bioplastic samples for 45 days were 12.44% and 28.07% for the bioplastic without and with tithonia extract, respectively. Complete biodegradation of the bioplastic predicted in 244 days and 200 days for the bioplastic without and with tithonia extract, respectively.

[Kata kunci : *Tithonia diversifolia*, biodegradability, bioplastic, plantation soil].

Introduction

The production of cocoa beans in Indonesia has decreased since 2010, although the area of cocoa plantation has increased. The lowest production in 2015 was 66,243 tons. Cocoa productivity has declined since 2000. Indonesia's cocoa productivity in 2000 was 724 kg/ha and decreased by 52% to 383 kg/ha in 2015 (Dirjenbun, 2016). The decline of the cocoa production can be accounted for by a number of factors, i.e.: decreasing soil fertility and organic matter content; and an increased incidence of pests/diseases, such as cocoa pod borer (CPB) caused by *Conopomorpha cramerella* and *Phytoptora* pod rod (McMahon *et al.*, 2015). The attack of CPB caused damage to 62.3% of the cocoa bean (Afrizon & Rosmana, 2002) and caused loss of the production up to 82.20% (Depparaba, 2002).

An effective control approach for CPB is cocoa pod sleeving using plastic or biodegradable

plastic (Saripah *et al.*, 2007; Rosmana *et al.*, 2013; Joseph *et al.*, 2017). This method is most effective when compared to other methods. Sleeving of the cocoa pod can suppress CPB attacks up to 85-95%. However, the use of plastic that cannot biodegradable in nature still allows the emergence of plastic waste. Use of the biodegradable plastic could be a potential technique to control CPB and save for the environment (Sembel *et al.*, 2011).

Our research team developed a bioplastic composite made of cellulose and starch and used to control CPB in cocoa plantation. The bioplastic enriched by an extract of *Tithonia diversifolia* as organic pesticide. The bioplastic was successfully made into sheet and bag. However, although all components in the bioplastic are biodegradable, but biodegradability of the bioplastic has not tested. Aims of this research is to test the biodegradability of the bioplastic in plantation soil as natural inoculum by measurement of carbon dioxide released during biodegradation the test. Degradation of the bioplastic in aerobic condition by microorganism would release carbon dioxide, water and heat as degradation result. The carbon dioxide was measured by titration methods.

Material and Method

Bioplastic samples

Bioplastics used in this experiment were a composite of cellulose fiber, cassava starch as matrix biopolymer and glycerol as plasticizer. The cellulose fiber was extracted and purified from cocoa pod husk by soda caustic methods (Isroi *et al.*, 2017). Ratio between cellulose and starch was 25:75 by weight. Bioplastic made by casting methods in acrylic plate 20 cm x 20 cm. Other bioplastic sample was a bioplastic enriched by *Tithonia diversifolia* extract. The plant extract

prepared by pilot scale extractor and using water as solvent. The plant extract added in the bioplastic formulation by 10% from the total volume. All the samples were cut into 1 cm to 1 cm pieces. The all samples keep in desiccator to maintain the humidity before use.

Plantation soil

The inoculum used in the biodegradation test was local plantation soil, Dramaga, Bogor West Java, collected from the top soil 5 cm to 10 cm in deep. The soil was screened to remove debris and an organic material. Characteristics of the soil inoculum was shown in Tabel 1.

Biodegradation test

Biodegradation of biomass material, such as bioplastic, by microorganism in aerobic condition released carbon dioxide, water and heat. Amount of the carbon dioxide proportional to degradation of the bioplastic. Biodegradation test was conducted in 300 ml glass bottle arrangement as Figure 1 according to the reference (Khaswar *et al.*, 2008). A quantity of 100 g of the plantation soil was placed into the samples bottle. Each bioplastic and reference plastics samples (± 0.1 g) were placed and buried into the soil. A bottle without bioplastic or plastic samples was used as control test to corrected the carbon dioxide released during the test. Carbon dioxide was channelled using tube to the other bottle with 50 mL of 0.1 N sodium hydroxide. The carbon dioxide would react with the sodium hydroxide and reduce the concentration of sodium hydroxide accordingly. All experiment held in duplo experiment and in room temperature (28°C) for 45 days. Every tree days the sodium hydroxide was replaced and analyzed for carbon dioxide.

Table 1. Characteristic of the soil inoculum used in the biodegradation test

Tabel 1. Karakteristik inokulum tanah yang digunakan dalam uji biodegradasi

Characteristics/Karakteristik	Value/Nilai
Total plate count/ <i>Jumlah total koloni (TPC) (cfu/g)</i>	21.3 x 10 ⁵
Water content/ <i>Kadar air (%)</i>	32.95
Total solid/ <i>Padatan total (%)</i>	67.05
C-organic content/ <i>Kandungan C-organik (%)</i>	12.28
Nitrogen content/ <i>Kandungan nitrogen (%)</i>	1.91
C/N ratio/ <i>Rasio C/N</i>	5.00
pH	6.0

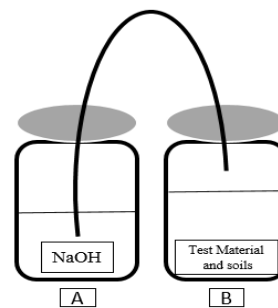


Figure 1. Experimental setup of the biodegradation test, (A) carbon dioxide trap with 0.1 N NaOH, (B) test material and plantation soil

Gambar 1. Bagan rancangan percobaan dari uji bioderabiodegradasi, (A) perangkap carbon dioksida yang berisi 0,1 N NaOH, (B) bahan uji dan tanah perkebunan

Carbon dioxide analysis

The carbon dioxide entrapped in the sodium hydroxide determined by titration with 0.1 N hydrochloric acid solution. Phenolphthalein (PP) used as first indicator and methyl orange as second indicator. Amount of hydrochloric acid needed to neutralized the solution into pink is proportional with the amount of sodium hydroxide after being reduced by the reaction with carbon dioxide releases during the biodegradation test

Results and Discussion

Bioplastic properties

Lignocellulose content of the cellulose fiber extracted from the cocoa pod shown in Table 2. The cellulose content was 50.58%. This cellulose content increasing two times than initial cocoa pod. Hemicellulose content was the highest lingo-cellulose in the cocoa pod (Daud *et al.*, 2013). Cellulose fiber extraction by soda caustic methods reduce the hemicellulose and extractive. The lignin content remains the same percentage as in the initial content. Cellulose content obtained in this research are similar as reported as by Hutomo *et al.*, (2012). Soda caustic methods are reduced more hemicellulose than others lignocellulose component. Dominant amount of the hemicellulose and reactivity of the hemi-cellulose to soda caustic could be cause of this result.

The bioplastic is a cellulose:starch based bioplastic and made by casting method. Glycerol are used as plasticizer. Bioplastic sheets and appearance of the bioplastics shown in Figure 2. Bioplastic without tithonia extract is more transparent and opaque than bioplastic enriched with tithonia extract. Bioplastic with tithonia extract is darker than the previous one. Adding tithonia extract was not affected thickness of the bioplastic. Thickness of these bioplastics are 90-110µm. Two physical properties of the bioplastics

are presented in Figure 3. Adding plant extract increased the WVTR (water vapour transmittance rate) of the bioplastic. Higher WVTR mean that the bioplastic absorbs more water from the environment. Increasing WVTR followed by significantly reducing tensile strength of the bioplastic as shown in the graphic.

Carbon dioxide released during the biodegradation test

Plantation soil rich in microorganism and the total colony was 21.3×10^5 colony per g soil including bacteria, fungi and actinomycetes. These microorganisms used organic carbon as energy source. In aerobic condition the organic material, such as bioplastics, would be bio-degraded into CO₂, H₂O and produce heat (Adhikari *et al.*, 2016). The carbon dioxide released are fluctuate time by time and shown in Figure 4. Carbon dioxide from the control experiment, without bioplastic samples, was carbon dioxide released from the original organic carbon source in the soil. Carbon dioxide released from the bioplastic experiments were sum of the carbon dioxide released from the bioplastic and from the original organic carbon source.

Table 2. Lignocellulose content of the cocoa pod cellulose fibre

Tabel 2. Kandungan lingoselulosa dari serat selulosa kulit buah kakao

Content/Kandungan	Percentage/Persentase (%)
Hot water soluble (HWS)/ Komponen Larut Air Panas	3.99
Cellulose/Selulosa	50.58
Hemicellulose/ Hemiselulosa	10.07
Lignin/Lignin	34.60
Ash/Abu	0.76



Figure 2. Bioplastic composite of cellulose from pod cacao and starch: (A) bioplastic without tithonia extract and (B) bioplastic enriched with tithonia extract

Gambar 2. Komposit bioplastic dari selulosa kulit buah kakao dan pati: (A) bioplastik tanpa penambahan ekstrak tithonia dan (B) bioplastik dengan penambahan ekstrak tithonia

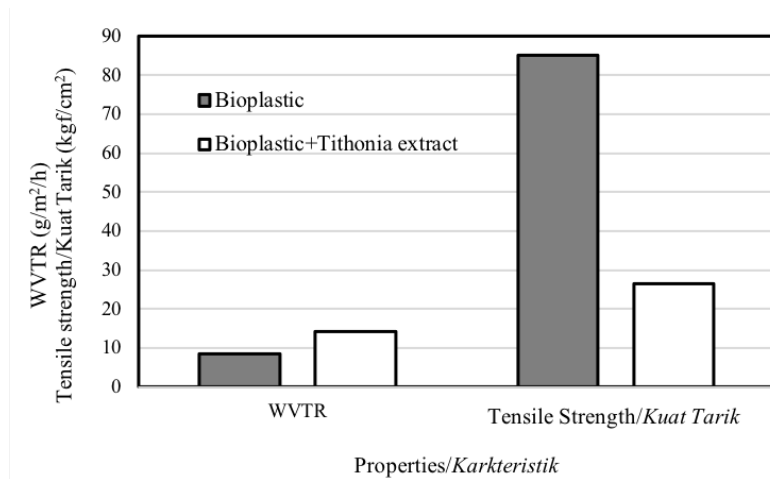


Figure 3. Water vapour transmittance rate (WVTR) and tensile strength of the bioplastic samples
 Gambar 3. Water vapour transmittance rate (WVTR) dan kuat tarik dari contoh bioplastik

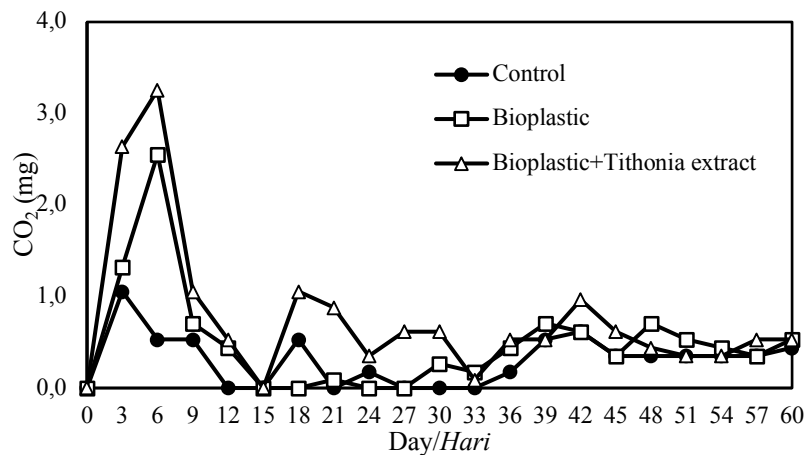


Figure 4. Carbon dioxide (mg) released during the biodegradation test
 Gambar 4. Karbon dioksida (mgr) yang dilepaskan selama uji biodegradasi

During the first six days, the microorganisms were very active to degrade the organic carbon as shown by high CO₂ released. Evolution of the CO₂ then gradually reduce and become more stable two weeks after incubation. Evolution of the CO₂ on the control experiment was the lowest. The CO₂ evolution from the bioplastic enriched with tithonia extract was higher than from the bioplastic without tithonia extract. Although tithonia extract are known as organic pesticide for some insect larvae (Otusanya & Ilori, 2012), but this plant extract not toxic for the microorganisms and could be degraded by microorganism. Content of the tithonia extract are flavonoid and terpenoid (Miranda *et al.*, 2015). The percentage content of the tithonia extract used in this study was 3.46% flavonoid and 0.05% tannin.

Biodegradation rate

Biodegradation of the bioplastic samples were represented as sum of the carbon dioxide released from the bioplastic samples and subtracted by control experiment. Biodegradation of the bioplastic samples are shown in Figure 5. In

accordance with the previous graphic, biodegradation of the bioplastic enriched with tithonia extract was higher than the bioplastic without plant extract. The bioplastic without plant extract initiated to biodegraded at the day three, then the biodegradation rate slower and become stable after three weeks. The bioplastic with plant extract already biodegraded from the beginning and continued until the end of the study. Total CO₂ released during the biodegradation test was 4.58 mg and 9.59 mg for the bioplastic without plant extract and enriched with plant extract, respectively.

Biodegradation rate of the bioplastic samples predicted by regression analysis of the data. Biodegradation rate of the bioplastic samples were 0.059 mg CO₂/day and 0.131 mg CO₂/day for the bioplastic without plant extract and enriched with plant extract, respectively. Its mean that the based bioplastic enriched with the plant extract are easier to biodegraded by microorganism than the bioplastic without the plant extract. By calculation, total degradation of the bioplastic samples were 428 days and 215 days, respectively. Resume of the biodegradation test presented in Table 3.

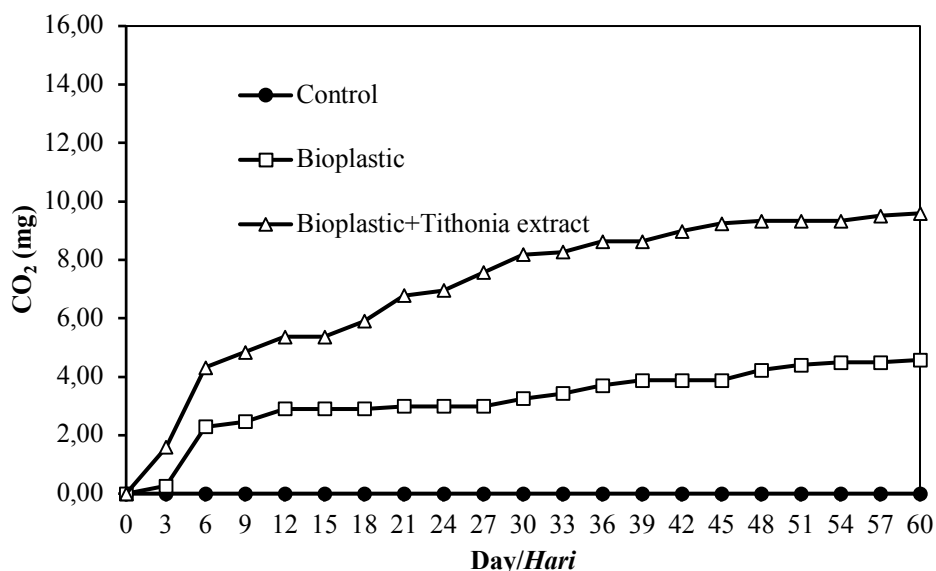


Figure 5. Total carbon dioxide (mg/day) released during the biodegradation test from the bioplastic samples corrected with the control experiment

Gambar 5. Karbon dioksida total (mgr/hari) yang dilepaskan selama uji biodegradasi untuk contoh bioplastik yang sudah dikoreksi dengan perlakuan kontrol

Table 3. Resume of the biodegradation test of the bioplastic samples

Tabel 3. Ringkasan hasil pengujian biodegradasi contoh bioplastik

Samples/Contoh	Theoretical CO ₂ /CO ₂ Teoritis (mg)	Biodegradation rate/Kecepatan biodegradasi (mg CO ₂ /day)	Prediction of the total biodegradation/Perkiraan waktu biodegradasi total (day)
Bioplastic/Bioplastik	26.626	0.059	428
Bioplastic+tithonia extract/Bioplastik+ekstrakt tithonia	31.370	0.131	215

Conclusion

Advantages of the bioplastic compared to the conventional plastic for controlling CPB are biodegradability in natural environment. This study is successfully shown the biodegradation of the bioplastic samples in plantation soil. All bioplastic samples could be biodegraded by microorganism as shown by CO₂ evolution during the test. Adding tithonia extract to the bioplastic formulation has benefit effect to the biodegradability of the bioplastic. In other hand, the plant extract could act as organic pesticide for the larvae of the CPB.

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