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ชื่อโครงการ	Biodegradation of PLA dip coated film under aerobic condition by measuring carbon dioxide evolved in soil.
ชื่อนิสิต	Mr. Phalod Towsiri
ภาควิชา	Environmental science
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คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

การย่อยสลายทางชีวภาพของแผ่นเคลือบฟิล์มพอลิแลคติคแอซิดภายใต้สภาวะที่มีออกซิเจนโดยการวัดปริมาณก๊าซคาร์บอนไดออกไซด์ที่เกิดขึ้นในดิน

นายพลช เถาว์ศิริ

โครงงานนี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรบัณฑิต

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Biodegradation of PLA dip coated film under aerobic condition by measuring carbon dioxide evolved in soil.

Mr. Phalod Towsiri

Senior Project Submitted in Partial Fulfillment of Requirements for the Bachelor's Degree in Environmental Science

Department of Environmental science

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Project Title	Biodegradation of PLA dip coated film under aerobic condition by measuring carbon dioxide evolved in soil.
By	Mr. Phalod Towsiri
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Project Advisor	Associate Professor Roongkan Nuisin, Ph.D

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of Requirements for the Bachelor's Degree.

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ชื่อโครงการ: การย่อยสลายทางชีวภาพของแผ่นเคลือบฟิล์มพอลิแลกติคแอซิดภายใต้สภาวะที่มีออกซิเจนโดยการวัดปริมาณก๊าซคาร์บอนไดออกไซด์ที่เกิดขึ้นในดิน

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บทคัดย่อ

ศึกษาการย่อยสลายทางชีวภาพของฟิล์มพอลิแลกติคแอซิดบริสุทธิ์ ชนิด 4042D และฟิล์มจุ่มเคลือบพอลิแลกติคแอซิดบนกระดาษโดยการวัดปริมาณก๊าซคาร์บอนไดออกไซด์ที่เกิดขึ้น เตรียมฟิล์มจุ่มเคลือบพอลิแลกติกแอซิด ที่มีระยะเวลาจุ่มเคลือบฟิล์มต่างกัน 3 ช่วงเวลา ได้แก่ 10, 20, 40 นาที การย่อยสลายทางชีวภาพดำเนินการโดยนำฟิล์มตัวอย่างมาฝังดินในขวดบ่มทางชีวภาพฝาปิด ติดตามการย่อยสลายทางชีวภาพของตัวอย่างเมื่อเวลาผ่านไป 0, 1, 3, 5, 7, 14, และ 28 วัน เมื่อสิ้นสุดระยะเวลาที่กำหนด พบว่าฟิล์มพอลิแลกติคแอซิดบริสุทธิ์มีผลการย่อยสลายทางชีวภาพสูงสุด คิดเป็น ร้อยละ 21.4 สำหรับฟิล์มจุ่มเคลือบพอลิแลกติคแอซิดบนกระดาษที่เวลาจุ่ม 10 20 และ 40 นาที มีการย่อยสลายทางชีวภาพร้อยละ 14.6 10.9 และ 9.6 ตามลำดับ

คำสำคัญ

การย่อยสลายทางชีวภาพ; ดิน; พอลิแลกติคแอซิด; ฟิล์มจุ่มเคลือบ

Project title: Biodegradation of PLA dip coated film under aerobic condition by measuring carbon dioxide evolved in soil

Department: Environmental science

Student: Phalod Towsiri

Advisor: Associate Professor Roongkan Nuisin, Ph.D

Abstract

This study is focused on pure poly(lactic acid) type 4042D film and poly(lactic acid) dip-coated film on paper by measuring CO_2 evolution. The study of pure poly(lactic acid) film and poly(lactic acid) dip-coated film on paper by measuring CO_2 evolution. Poly(lactic acid)/paper dip-coated film prepared from poly(lactic acid) in three different dip-coating periods;10, 20 and 40 minutes. The biodegradation was set up by buried test sample in soil within microcosm. The biodegradation of sample was followed over time 0, 1, 3, 5, 7, 14 and 28 days. It was found that pure poly(lactic acid) film has the highest biodegradability at 21.4%. For poly(lactic acid)/paper dip-coated film at 10, 20, and 40 minute dip-coating time that shown biodegradation at 14.6%, 10.9% and 9.6%, respectively.

Keyword

biodegradation; dip-coated film; poly(lactic acid); soil

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Phalod Towsiri

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ABBREVIATION

$Ba(OH)_2$	Barium Hydroxide
$Ba(OH)_2 \cdot 8H_2O$	Barium Hydroxide Octahydrate
CO_2	Carbon Dioxide
D_{10}	10 minutes dip-coated film
D_{20}	20 minutes dip-coated film
D_{40}	40 minutes dip-coated film
g	Gram
h	Hour
HCl	Hydrochloric Acid
L	Liter
g	Milligram
ml	Milliliter
mm	Millimeter
mm/s	Millimeter per second
M	Molar
PLA	Poly(lactic acid)
w/v	Weight by volume
g/mol	Gram by mole
cm^2	Square centimeter

CHAPTER 1

INTRODUCTION

It is an undeniable fact that plastics are commonly used in every sector of industrials for their goods and services, including commercial and agricultural sectors. Every year, each sector generates and eliminates a large amount of plastic wastes. the research showed that since 2015, only 21% of plastics produced were eliminated by incineration and recycling process. However, the others 79% were left behind in the environment (Viera *et al.*, 2020). The accumulated plastics from the environment then become a root cause due to the fact that the deterioration of plastic pollution causes worldwide adverse effects to both terrestrial and aquatic environments (Emadian *et al.*, 2017). For instance, the plastics induce carbon dioxide emission to the environment as well as harm the wild-life ecosystem once they are contaminated with soil or water sources. Since most plastics are synthesized by petroleum-based polymers such as polyethylene, polypropylene or polyester, which made the plastic to become highly durable to the environment (Ghosh *et al.*, 2013). In addition, they are extremely slow degraded within the normal condition and hence the plastics are considered as highly resistant to the environment. Taking this into account, the plastics pollution must be emphasized immediately.

In Thailand, single-used plastics are extensively used in daily-life occurring an environmental issue, as most of single-used plastics are non-biodegradable. Biodegradable plastic is another option that helps to reduce the occurring issue. Due to their constituents, most biodegradable plastics are synthesized by bio-based polymers from renewable sources, which can decompose into water, carbon dioxide (CO_2) and biomass. Many companies such as, PTTGC, SCG have become more aware of the plastic pollution, hence, poly(lactic acid) (PLA) is introduced to replace the non-biodegradable plastics. Poly(lactic acid) are aliphatic polyester made up of lactic acid as shown in Figure 1.1. Lactic acid is chemical products which produced from agriculture products such as corn, cassava or sugarcane. PLA are considered as one of the most widespread biodegradable polymers. Owing to their suitable physical properties, PLA is widely used in many sectors medical, agricultural, automotive and

food packaging. PLA dip-coated film is one of PLA products that can be seen generally in form of paper cup. From environmental awareness, PLA paper cup is widely used in place of single-used plastic cups due to the properties that can be degraded via a biological process known as biodegradation (Gere & Czigany, 2020; Hu *et al.*, 2016).

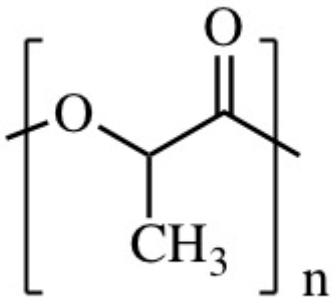

Figure 1.1 Chemical structure of poly(lactic acid)

Biodegradability has been considered an important attribute for molecular structure. There are three stages of PLA biodegradation process. First stage occur outside the microorganism, the extracellular enzyme such as PLA-depolymerase or proteases degrade PLA plastic into smaller structure, oligomers and monomers (Apinya *et al.*, 2015). Next, the beginning of phase 2 is when constituent elements which pass through the cell membrane and are metabolized becoming part of the biochemistry of the microorganisms and of the living mass. The final degradation of the metabolites involves an oxidation process that requires oxygen and leads to the evolution of CO_2 (Tosin *et al.*, 2019). Laboratory test methods for investigating and monitoring the biodegradation processes have been developed. The principal groups of measuring can be divided as follows: direct measurement of sample concentrations and indirect measurement via conversion process into products such as CO_2 evolution, total organic carbon content (Tosin *et al.*, 2019).

To follow CO_2 evolution, microorganism respiration is begun in the microrsm which is the closed system. CO_2 generated in microrsm is entrapped with CO_2 trapping solution. The amount of CO_2 produced in the microrsm is calculated by titration method. The biodegradation behavior of test sample can be studied by the quantification of CO_2 evolved from different samples and their carbon content.

This experiment was focused on the biodegradation of PLA/paper dip coated film buried in soil. Biodegradation was followed by CO_2 evolution according to the standard test method ASTM D 5988 – 12. The effects of PLA/paper dip-coating times and soil buried period were studied. Theoretical CO_2 was calculated to determine the biodegradation percentage of each different sample types.

1.1 Objectives

1. To determine the disintegration of PLA/paper dip-coated film in soil by measuring carbon dioxide evolution.
2. To aspect the biodegradation behavior of different dipping period of PLA/paper dip-coated film in soil.

1.2 Scope of the study

1. Poly(lactic acid) in this study is NatureWorks® poly(lactic acid) polymer 4042D produced from NatureWorks LLC, USA
2. The biodegradation test is followed up by carbon dioxide evolution of sample in range of 0 to 28 days.

1.3 Expected benefits

1. Appropriate dip-coating time for effective biodegrade of PLA/paper dip-coated film in soil.

CHAPTER 2

LITERATURE REVIEW

2.1 Poly(lactic acid)

2.1.1 Chemical structure and properties

Poly(lactic acid) (PLA) is biodegradable aliphatic polyester synthesized from which have chemical formula as $(C_3H_4O_2)_n$. Lactic acid, the monomer of PLA, is the by-product of glucose fermentation (the best route to obtain pure isomers desired for polymerization) in renewable resources such as corn, cassava, sugarcane (de Vlieger, 2003). In general, Lactic acid is formed in two optically active stereoisomer; D-lactic acid and L-lactic acid. Two molecule of LA combined to lactide which has three different forms of stereoisomer; L-lactide, D-lactide and D,L-lactide as shown in **Figure 2.1**.

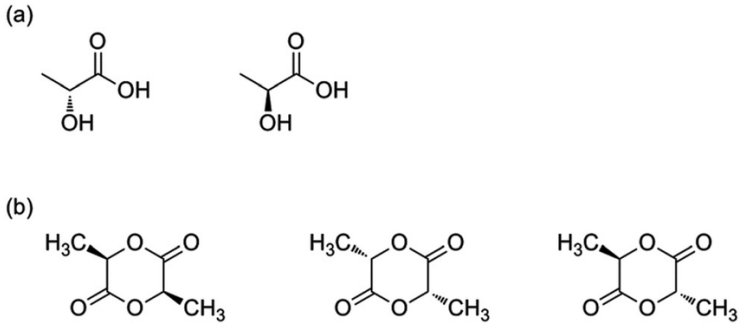

Figure 2.1 (a) Lactic acid structure; L-lactic acid and D-lactic acid, (b) Lactide structure; L-lactide, D-lactide and D,L-lactide (Corneillie & Smet, 2014)

PLA can be produced through three different processes, direct polycondensation, two steps polymerizate ion and ring-opening polymerization as shown in **Figure 2.2**. Normally, PLA which used in commercials (i.e., NatureWorks® poly(lactic acid) polymer 4042D) are synthesized from L-lactide or racemic D,L-lactide. L-lactide and D-lactide are semicrystalline, high tension strength and low extension with glass transition temperature (T_g) of approximately 63°C, while D,L-lactide is amorphous and T_g is approximately

55°C (Burg, 2014; Tokiwa & Calabia, 2006). Thus, PLA properties are depended on the ratio between this two forms isomer, lead to the wide-range of applications (de Vlieger, 2003; Hu *et al.*, 2016).

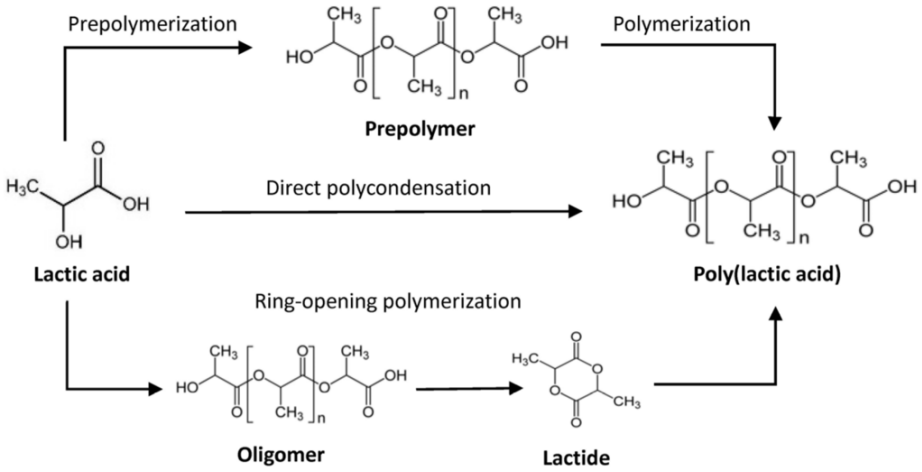

Figure 2.2 Poly(lactic acid) synthesized pathways (Luyt & Malik, 2019).

2.1.2 PLA applications

Due to the other properties such as low molecular weight, environmental friendly, biodegradability, high physical strength, transparency and easy processability; PLA is considered as the most popular biodegradable plastic applicated in various fields such as medical, agricultural, automotive, or food packaging (Hu *et al.*, 2016; Qi *et al.*, 2017). PLA is famous for packaging application (e.g., cups, films or sheets) because it is designed for food contact, the residue PLA is degraded into lactic acid which occurred normally in food and body (de Vlieger, 2003).

2.1.3 Soil biodegradation of poly(lactic acid)

In an aerobic conditions, PLA can be degraded by living organisms. Thus, the completed degradation takes long period, about several month up to a years related to polymeric factors that involved the biodegradability (e.g., molecular weight, crystallinity, glass transition temperature, melting temperature, surface area, hydrophobic and hydrophilic properties) and environmental conditions. Carbon dioxide, methane and biomass are represented as the complete biodegradation processing (Qi *et al.*, 2017).

PLA biodegradation mechanism generally consist of three principle stages. First stage started outside the microorganism, PLA debris in landfill or which contaminated in soil contacted with extracellular enzyme occurring the deterioration of its surface. The deterioration of PLA is mainly caused by the lack of the main chain or the side chain of the molecules. The depolymerases attack to intramolecular ester links, the ester bonds are cleaved and the high-molecular weight molecules are split into low-molecular weight molecules (i.e., monomers, oligomers). Followed by assimilation of microorganisms through their cell membrane. The second stage is beginning when the small molecules are metabolized by biochemical processes change into cell biomass. In the last stage, organic carbons are decomposed by microorganism to gaseous compounds (i.e., CO_2) and water through mineralization as shown in Equation 2.1 (Chinaglia *et al.*, 2018; Kim *et al.*, 2017; Tosin *et al.*, 2019).

$$C_{polymers} + O_2 \rightarrow C_{biomass} \rightarrow CO_2 + H_2O \qquad (2.1)$$

2.2 Biodegradation

Biodegradation, the important biological process of which transform or eliminate of the organic compound by microorganism activities to remedy the contaminated soil or aquatic environment. Biodegradation process are classified into two board categories; (1) aerobic biodegradation or (2) anaerobic biodegradation. The different between two categories is that aerobic process required oxygen as the substrate, by-products from the reaction are CO_2, water and the biomass, while anaerobic process do not need oxygen and the reaction products are same to aerobic process just only methane added. The biodegradability is limited by the environmental conditions (e.g., soil pH, soil moisture, temperature, nutrients), microorganisms and compound structure properties (Speight, 2015). The mechanism of aerobic biodegradation is following by three main phases; depolymerization, bio assimilation and mineralization (Tosin *et al.*, 2019).

2.3 Carbon dioxide evolution in soil

Carbon dioxide evolution is the method to study the microbiological activities in soil by measuring the CO_2 generated from biodegradation process. Because of, CO_2 is the major product of aerobic microbial decomposition, CO_2 become one of the parameters for determine biodegradation percentage and rate of biodegradation followed by CO_2 evolution test method. This standard method is designed to measure CO_2 generated from biodegradable plastic contacted with soil in an aerobic condition. To determine the CO_2 evolution, the experiments require closed system model likes, microrsm. CO_2 generated in the microrsm is captured by the trapping solutions for example, $Ba(OH)_2$ as shown in Equation 2.2;

$$Ba(OH)_{2\,(aq)} + CO_{2\,(g)} \rightarrow BaCO_{3\,(s)} + H_2O_{(l)} \qquad (2.2),$$

CO_2 gases react with $Ba(OH)_2$ solution to produce $BaCO_3$, the white solid that is insoluble in water and soluble in most acid, and water. Then, the remained $Ba(OH)_2$ is titrated with HCl as Equation 2.3;

$$Ba(OH)_{2\,(aq)} + 2HCl_{\,(aq)} \rightarrow BaCl_{2\,(aq)} + 2H_2O_{(l)} \qquad (2.3)$$

The CO_2 evolution in the microrsm can be determined by stoichiometry from the amount of HCl used for titration. The biodegradation percentage is calculated from the amount of evolved CO_2 and the amount of carbon content in material (Chinaglia *et al.*, 2018).

CHAPTER 3

MATERIALS AND METHODS

3.1 Chemicals and Reagents

- Poly(lactic acid) (NatureWorks® PLA polymer 4042D, NatureWorks LLC, USA)
- Filter papers (Whatman No.5, Cytiva, USA)
- Dichloromethane (CH_2Cl_2, M.W. = 84.93 g/mol, Analytic grade, Mallinckrodt, USA)
- Hydrochloric acid (HCl, M.W. = 36.46 g/mol, Analytic grade, Qrec, New Zealand)
- Barium hydroxide ($Ba(OH)_2 \cdot 8H_2O$, M.W. = 315.47 g/mol, Analytic grade, Ajax finechem, Australia)
- Phenolphthalein
- Deionized water
- Soil
- Standard sieve
- Screw cap glass bottle

3.2 Equipment

- Incubator (Model 600, Memmert, Germany)

3.3 Preparing of PLA film

The test material in this study was NatureWorks® poly(lactic acid) polymer 4042D produced from NatureWorks LLC, USA in form of pellets and the density is 1.25 g/ml. PLA pellets was dissolved in dichloromethane (10%w/v) utilizing a magnetic stirrer for 3 h at 25°C until a complete homogenous were formed. 20 ml of PLA solution was poured on flat glass plate and left in room temperature over night. The set PLA films were cut into pieces about 1×1 cm^2 (Hossain *et al*., 2018)

3.4 Dip-coating process

The filter paper (Whatman No.5) were used as a core for dip-coating. Paper were dipped in 10% PLA solution in various desired dipping periods; 10, 20 and 40 minutes. When time has reached, PLA dip-coated papers were hung in fume hood over night at room temperature. Completed PLA dip-coated papers were cut into pieces about 1×1 cm^2, determine the percentage of PLA dip coated film by weight changed.

3.5 Soil characterization

Soil media is prepared by sieved to smaller than 2 mm by standard sieve and determined properties (Table 3.1).

Table 3.1 Soil analysis method

Parameter	Methods	References
Soil texture	Soil sieve method	(Land Development Department, 2018)
pH	pH meter	(Land Development Department, 2018)
Soil moisture	Gravimetric method	(Land Development Department, 2018)
Soil organic matter	Walkley Black method	(Land Development Department, 2018)
Soil-water holding capacity	Volumetric method	(Land Development Department, 2018)
Total nitrogen	Kjeldahl method	(Land Development Department, 2018)

3.6 Biodegradation test

Rate of biodegradation was determined by means of respirometric tests, in accordance with the ASTM D 5989 – 12 test method, based on the measurement of carbon dioxide production. The screw cap glass bottles were used as the microrsm. Each of bottle was filled with 30g of soil. The soil moisture was adjusted to 8.5% which is about 50% of soil water holding capacity. The test samples was set up with blank bottle and with the reference (1×1 cm^2 Filter paper) for three replicates as shown in Table 3.2 (Tosin *et al*., 2019). The samples were divided into two types; (1) PLA films

and (2) PLA dip-coated films which prepared in three different dipping periods. Filter paper was used as the reference.

Table 3.2 Biodegradation test samples

Label	Condition
Day_B.1	Soil without treatment (Blank)
Day_B.2	Soil without treatment (Blank)
Day_B.3	Soil without treatment (Blank)
Day_C.1	Filter paper
Day_C.2	Filter paper
Day_C.3	Filter paper
Day_P.1	PLA film
Day_P.2	PLA film
Day_P.3	PLA film
Day_D10.1	Ten minutes dip-coated film
Day_D10.2	Ten minutes dip-coated film
Day_D10.3	Ten minutes dip-coated film
Day_D20.1	Twenty minutes dip-coated film
Day_D20.2	Twenty minutes dip-coated film
Day_D20.3	Twenty minutes dip-coated film
Day_D40.1	Forty minutes dip-coated film
Day_D40.2	Forty minutes dip-coated film
Day_D40.3	Forty minutes dip-coated film

Biodegradation test was set up as shown in Figure 3.1 and Figure 3.2.

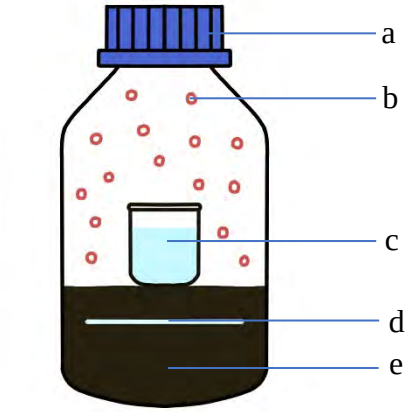

Figure 3.1 The microrsm to determine rate of biodegradation

where; a is screw cap glass bottle as microrsm

b is carbon dioxide generated in the microrsm

c is vial bottle filled with carbon dioxide trapping solution

d is test sample

e is soil

Figure 3.2 Biodegradation microrsm set-up

A 20 ml vial bottle was filled with 20ml of 0.08 M $Ba(OH)_2$ and placed on top of soil. Each set of microrsms were left in the incubator at 37±2 °C for 1, 3, 5, 7,14 and 28 days. Thirty gram of soil was added into the screw cap glass bottle and soil moisture content was adjusted to be equal to 8.5% (50% of water holding capacity). When the time was reached, vials were taken off from the microrsm, pipetted 5 ml of $Ba(OH)_2$ into Erlenmeyer flask and added 15 ml deionized water. The solution was titrated with 0.030 M HCl and phenolphthalein as the indicator. Rate of biodegradation was determined by these following Equations 3.1;

$$T = T_b - T_s \qquad (3.1),$$

where; T is the calculated amount of HCl needed to titrate the CO_2 generated from the sample (ml).

T_b is the amount of HCl used to titrate the blank bottle (ml).

T_s is amount of HCl used to titrate the bottle containing soil and test sample (ml).

Then, amount of CO_2 evolution was calculated by Equation 3.2 (Al-Salem *et al.*, 2019).

$$CO_{2\,(P.)} = T \times 0.03 \times 22 \times \frac{L_{all}}{L_{pull}} \qquad (3.2),$$

where; $CO_{2\,(P.)}$ is amount of CO_2 evolved from test sample biodegraded (mg)

L_{all} is quantitative of $Ba(OH_2)$ in vial (ml)

L_{pull} is quantitative of $Ba(OH_2)$ which pulled for titration (ml)

Next, the theoretical CO_2 amount was calculated from carbon content of sample by stoichiometry as shown in Equation 3.3;

$$CO_{2\,(Th.)} = m \times C \times \frac{44}{12} \quad (3.3),$$

where; $CO_{2\,(Th.)}$ is amount of CO_2 which can be generated from each test sample (mg)

m is weight of test sample (mg)

C is carbon content of test sample (%)

Afterward, the biodegradation percentage (D) was calculated from the ratio between the CO_2 produced and the theoretical CO_2 as shown in Equation 3.4;

$$D = \frac{CO_{2\,(P.)}}{CO_{2\,(Th.)}} \times 100 \quad (3.4)$$

where; D is the percentage of biodegradation (%). Finally, the data was analyzed and plotted using the statistic tools.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Soil properties characterization

Soil media was collected from cassava plantation area in Rayong on 7 November 2019. Soil was sieved to 2 mm. particle size. From soil analytical by the standard methods as mentioned in Table 3.1 (Land Development Department, 2018). The results showed that soil texture was sandy loam consist with 70.8% of sand, 11.7% of silt and 17.5% of clay. Soil contained organic matter 0.81%. The soil moisture was 6.4%. Soil-water holding capacity was 17.1% and soil pH was neutral at 6.83.

4.2 PLA dip coated film

The PLA/paper dip-coating film was examined. It was found that PLA coated percentage of each types of sample were shown in Table 4.1. PLA was well-coated in less time period of dipping process. Ten minutes dip-coated film (sample D_{10}) has PLA dip-coated percentage about 36.6%. Twenty minutes dip-coated film (sample D_{20}) and Forty minutes dip-coated film (sample D_{40}) have the PLA percentage about 33.9% and 31.8% respectively. This was because of the withdrawal velocity. Fang *et al.* (2008) reported that at PLA concentration was 15%, if the withdrawal velocity was more than 16 mm/s the average thickness of PLA/paper dip-coating film presented as the slight decline curve and PLA/paper dip-coated film thickness was directed variation to PLA weight (Fang *et al.*, 2008)

Table 4.1 Poly(lactic acid) coated percentage of each dipping periods

Sample	Paper weight (g)		PLA weight (g)	PLA onto paper (%)
	Before coated	After coated		
D_{10}	1.2410	1.9575	0.7165	36.6
D_{20}	1.2675	1.9187	0.6512	33.9
D_{40}	1.2591	1.8463	0.5871	31.8

4.3 Carbon dioxide evolution of poly(lactic acid) samples

Theoretical carbon dioxide (Th_{CO2}) of samples were calculated by carbon content of sample and their weight. Carbon content factor of each sample was determined from their composition between cellulose and PLA. All of samples were weight by 4-digit analytical balance before started the biodegradation test as shown in Table 4.2. The Th_{CO2} was shown in Table 4.3.

Soil buried time (Day)	Sample	Average sample weight (g) ± SD
1	Filter paper	0.0103 ± 0.0001
	PLA	0.0108 ± 0.0012
	D_{10}	0.0161 ± 0.0004
	D_{20}	0.0152 ± 0.0009
	D_{40}	0.0152 ± 0.0005
3	Filter paper	0.0100 ± 0.0005
	PLA	0.0094 ± 0.0003
	D_{10}	0.0159 ± 0.0009
	D_{20}	0.0161 ± 0.0014
	D_{40}	0.0156 ± 0.0009
5	Filter paper	0.0101 ± 0.0001
	PLA	0.0103 ± 0.0014
	D_{10}	0.0160 ± 0.0004
	D_{20}	0.0146 ± 0.0013
	D_{40}	0.0154 ± 0.0013
7	Filter paper	0.0102 ± 0.0002
	PLA	0.0116 ± 0.0011
	D_{10}	0.0155 ± 0.0006
	D_{20}	0.0161 ± 0.0006
	D_{40}	0.0160 ± 0.0007
14	Filter paper	0.0103 ± 0.0003
	PLA	0.0120 ± 0.0010
	D_{10}	0.0163 ± 0.0006
	D_{20}	0.0151 ± 0.0013
	D_{40}	0.0148 ± 0.0004
28	Filter paper	0.0105 ± 0.0002
	PLA	0.0124 ± 0.0021
	D_{10}	0.0161 ± 0.0002
	D_{20}	0.0149 ± 0.0007
	D_{40}	0.0157 ± 0.0019

Table 4.2 Sample weight in each set of microcosm

Table 4.3 Theoretical CO_2 of sample in each soil buried time

Sample	Composition (%)		Carbon content factor	Soil buried time (Day)						
				0	1	3	5	7	14	28
	PLA	Filter paper (Cellulose)		Theoretical CO_2 (mg)						
Filter paper	0.00	100.00	1.6133	0.00	16.58	16.06	16.31	16.47	16.60	16.98
PLA	100.00	0.00	1.8333	0.00	19.88	17.17	18.94	21.27	22.04	22.81
D_{10}	36.60	63.40	1.6939	0.00	27.27	26.91	27.12	26.24	27.67	27.29
D_{20}	33.94	66.06	1.6880	0.00	25.70	27.14	24.61	27.14	25.53	25.15
D_{40}	31.80	68.20	1.6833	0.00	25.57	26.18	25.87	26.91	24.86	26.39

The CO_2 evolution was determined from the titration results. It presented that filter paper reaches the highest CO_2 production. Filter paper generated 6.1424 ± 0.2967 mg of CO_2 within 28 days buried in soil. The component of filter paper was cellulose which is a linear polymer of glucose. Although, cellulose was formed in crystalline microfibrils that was resistant to hydrolysis, almost microorganisms able to degrade cellulose. With their hydrophilic -OH group, caused cellulose had highest amount of CO_2 production. (Béguin & Aubert, 1994; Kalita *et al.*, 2020). The biodegradation of filter paper confirmed that microorganism in soil were active. The biodegradation of PLA film was found that the sample started the biodegradation at the first day of soil buried. In the day 1 and 3, CO_2 produced from PLA was nearly to the filter paper. After that, PLA degradation went down moderately but also still the uptrend. At day 28, PLA generated 4.8840 ± 0.4059 mg of CO_2. Due to PLA structure that is aliphatic polyester, the bond strength of ester bond in PLA was stronger than glycosidic bond caused it harder to break and resulted the lower CO_2 quantitative than filter paper produced. Followed up by D_{10}, D_{20}, D_{40} that produced 3.9776 ± 0.1718 mg CO_2, 2.7456 ± 0.1613 mg CO_2 and 2.5432 ± 0.1397 mg CO_2, respectively. CO_2 evolution trends were slightly uptrend. The results showed that proportion of CO_2 produced from PLA and PLA/paper dip-coated films were related to the PLA percentage of each type of samples. PLA film contained the most of PLA molecules in 4 type of test samples (PLA, D_{10}, D_{20}, D_{40}). It can be determined that the soil was rich of microbial generated protease and lipase which represent the ability to degrade PLA. The result reported that as much of the amount of PLA molecules show as much of CO_2 evolution from biodegradation as shown in Table 4.4 and Figure 4.1.

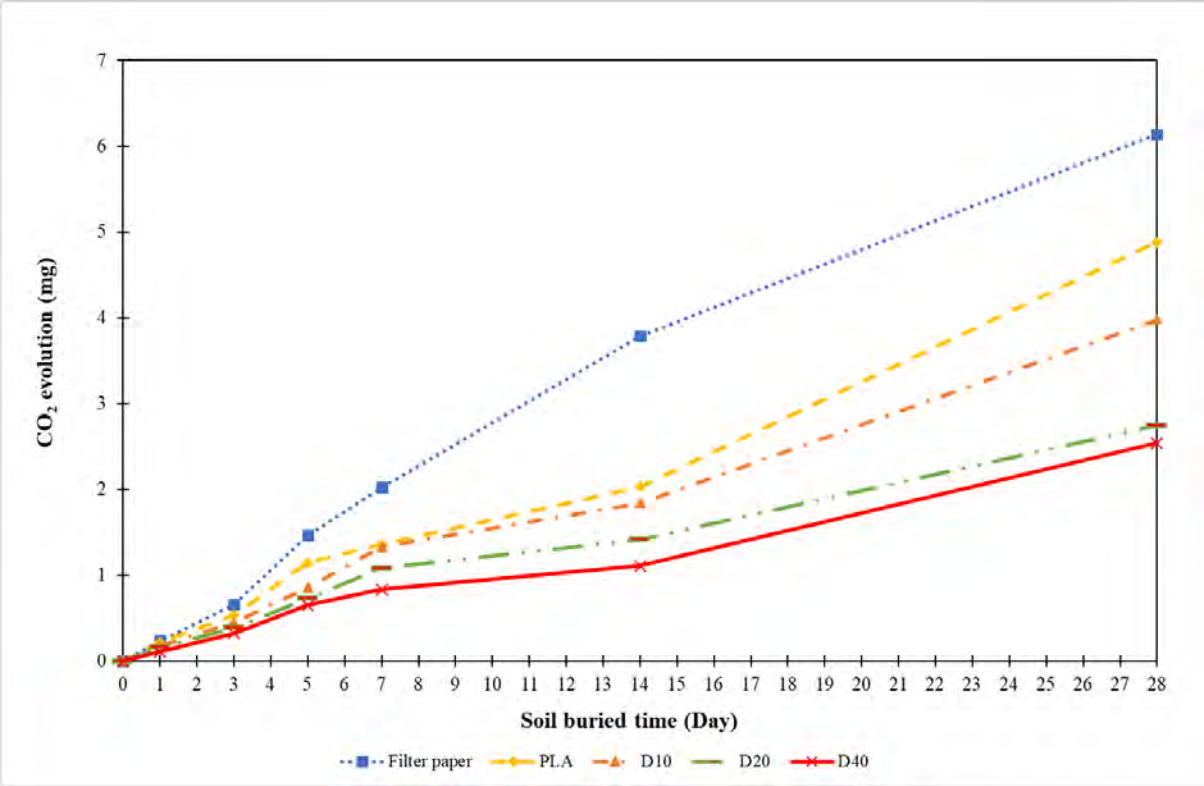

Figure 4.1 CO_2 generated in the microrsm of sample as function of soil buried time (each curve represented as average of three replicates)

Table 4.4 CO_2 evolution of the sample in different soil buried time

Sample	Soil buried time (Day)						
	0	1	3	5	7	14	28
	CO_2 evolution (mg)						
Filter paper	0.0000	0.2323 ± 0.0161	0.6600 ± 0.0528	1.4630 ± 0.1008	2.0240 ± 0.1873	3.7840 ± 0.1697	6.1424 ± 0.2967
PLA	0.0000	0.2182 ± 0.0211	0.5368 ± 0.0403	1.1440 ± 0.0873	1.3552 ± 0.0762	2.0328 ± 0.1355	4.8840 ± 0.4059
D_{10}	0.0000	0.1760 ± 0.0211	0.4488 ± 0.0528	0.8580 ± 0.1061	1.3288 ± 0.1758	1.8392 ± 0.1210	3.9776 ± 0.1718
D_{20}	0.0000	0.1584 ± 0.0220	0.3872 ± 0.0403	0.7260 ± 0.1159	1.0824 ± 0.0952	1.4168 ± 0.0952	2.7456 ± 0.1613
D_{40}	0.0000	0.1091 ± 0.0220	0.3256 ± 0.0403	0.6490 ± 0.0873	0.8360 ± 0.0664	1.1088 ± 0.0927	2.5432 ± 0.1397

4.4 Biodegradation percentage of PLA/paper-dip coated film

The biodegradation percentage of testing samples was calculated from the fraction of quantitative of CO_2 produced from biodegradation process and the theoretical carbon dioxide of the sample. The data of biodegradation percentage was complied with CO_2 evolution plot, the results of biodegradation percentage were reported in Table 4.5 and Figure 4.2. It showed that filter paper did the most biodegradation percentage that was 36.2 ± 1.75%. Filter paper degradation was slightly lower than Tosin *et al.* (2019) experiment that resulted about 65% biodegradation of microcrystalline cellulose in form of powder in same period because the powder has more surface area than sheet so the microorganism contacted surface was more than the sheet of filter paper (Tosin *et al.*, 2019). In addition, soil organic matter was 0.81% which classified in low level, caused the efficiency of microorganism activities dropped. Next, the PLA degradation percentage was up to 21.4 ± 1.78% close to Castro-Aguirre *et al*. (2018) reported that PLA film degraded about 25% at 30 composting days (Castro-Aguirre *et al*., 2018). When, D_{10} was degraded 14.6 ± 0.63% after 28 days. Followed up by D_{20} and D_{40} which had the biodegradation percentage equal to 10.9 ± 0.64% and 9.6 ± 0.53% respectively.

As known that D_{10}, D_{20} and D_{40} is PLA/paper dip-coated film, their components were only 30% of PLA and other was filter paper. At day 28, they showed about 10% degraded but PLA percentage coated on paper was about 30%. After the PLA coated was almost degraded they will show the rate of degradation like the filter paper and degrade faster.

These results showed that dip-coating time was affected to their biodegradation behavior. Within 28 days, PLA film had more efficiency to biodegrade than product of PLA/paper dip-coated film. Ten minutes of dip-coating process presented the best biodegrade behavior compared to other PLA/paper dip-coated film. Followed up by D_{20} and D_{40} respectively.

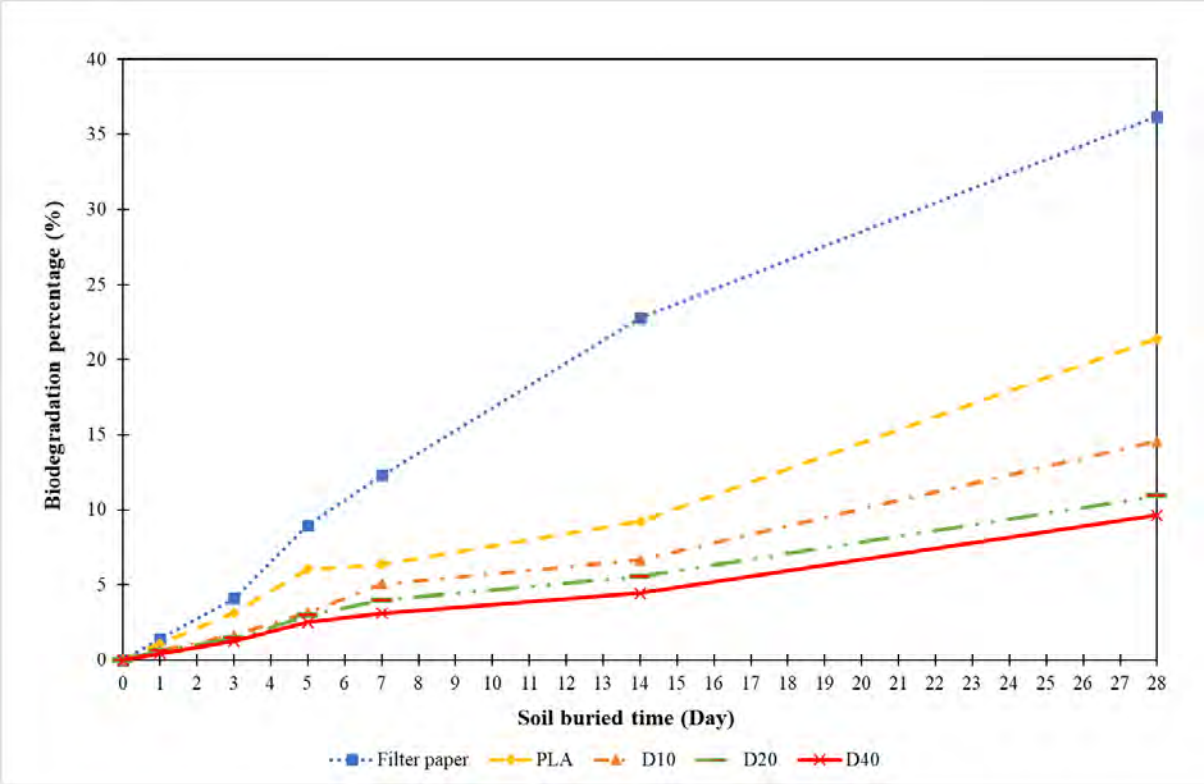

Figure 4.2 Biodegradation percentage of sample as function of soil buried time (each curve represented as average of three replicates)

Table 4.5 Biodegradation percentage of the sample in different soil buried time

Sample	Soil buried time (Day)						
	0	1	3	5	7	14	28
	Biodegradation percentage (%)						
Filter paper	0.00	1.40 ± 0.10	4.11 ± 0.33	8.97 ± 0.62	12.29 ± 1.14	22.80 ± 1.02	36.18 ± 1.75
PLA	0.00	1.10 ± 0.11	3.13 ± 0.23	6.04 ± 0.46	6.37 ± 0.36	9.22 ± 0.61	21.41 ± 1.78
D_{10}	0.00	0.65 ± 0.08	1.67 ± 0.20	3.16 ± 0.39	5.06 ± 0.67	6.65 ± 0.44	14.58 ± 0.63
D_{20}	0.00	0.62 ± 0.09	1.43 ± 0.15	2.95 ± 0.47	3.99 ± 0.35	5.55 ± 0.37	10.92 ± 0.64
D_{40}	0.00	0.43 ± 0.09	1.24 ± 0.15	2.51 ± 0.34	3.11 ± 0.25	4.46 ± 0.37	9.64 ± 0.53

CHAPTER 5

CONCLUSION

The biodegradation PLA and three different type of PLA/paper dip-coated films in soil followed up by CO_2 evolution during 0 to 28 day. After 28 day of test, the result shown that CO_2 evolution was go along with biodegradation percentage of samples. Filter paper which used as reference produced the most quantitative of CO_2, 6.1424 ± 0.2967 mg, compared to biodegradation percentage that equal to 36.18 ± 1.75%. Next, pure PLA film represented the second amount of CO_2 and biodegradation percentage which equal to 4.8840 ± 0.4059 and 21.41 ± 1.78% respectively. Three different types of PLA/paper dip-coated film were preparing via dip-coating process in different interval time, ten minutes, twenty minutes and forty minutes. The result of film preparation showed that different dip-coating time, the proportion of PLA coated onto the film surface were obtained. It was found that the percentage of PLA coating were 36.6%, 33.9%, and 31.8% for D_{10}, D_{20}, and D_{40}, respectively. The D_{10} was presented the highest CO_2 produced and biodegradation percentage compared with other dip-coated film, equal to 3.9776 ± 0.1718 mg and 14.6 ± 0.63%. The D_{20} was produced 2.7456 ± 0.1613 mg CO_2 and had the biodegradation percentage at day 28 about 10.9 ± 0.64%. Last, D_{40} degradation generated 2.5432 ± 0.1397mg CO_2 which mean that biodegradation percentage was 9.6 ± 0.53%.

The experiment showed that in range of biodegradation day 0 to 28, PLA/paper dip-coated film biodegradation behavior was related to their proportion of PLA. Pure PLA which contain highest amount of PLA molecule caused PLA had high biodegradation percentage followed up by D_{10}, D_{20} and D_{40} respectively.

REFERENCES

Al-Salem, S., Al-Nasser, A., Sultan, H., & Karam, H. (2019). In *situ* aerobic biodegradation in soil contact using the standard test method of ASTM D 5988-12. *IOP Conference Series: Earth and Environmental Science, 291*, 012011.

Apinya, T., Sombatsompop, N., & Prapagdee, B. (2015). Selection of a *pseudonocardia sp.* RM423 that accelerates the biodegradation of poly(lactic) acid in submerged cultures and in soil microcosms. *International Biodeterioration & Biodegradation, 99*, 23-30.

ASTM International, W. C., PA. (2012). ASTM D5988-12. Standard test method for determining aerobic biodegradation of plastic materials in soil.

Béguin, P., & Aubert, J.-P. (1994). The biological degradation of cellulose. *FEMS Microbiology Reviews, 13*(1), 25-58.

Burg, K. (2014). Poly(α-ester)s. In *Natural and Synthetic Biomedical Polymers*, Kumbar, S. G., Laurencin, C. T. & Deng, M. (Eds.), (pp. 115-121). Oxford: Elsevier.

Castro-Aguirre, E., Auras, R., Selke, S., Rubino, M., & Marsh, T. (2018). Enhancing the biodegradation rate of poly(lactic acid) films and PLA bio-nanocomposites in simulated composting through bioaugmentation. *Polymer Degradation and Stability, 154*, 46-54.

Chinaglia, S., Tosin, M., & Degli-Innocenti, F. (2018). Biodegradation rate of biodegradable plastics at molecular level. *Polymer Degradation and Stability, 147*, 237-244.

Corneillie, S., & Smet, M. (2014). PLA architectures: The role of branching. *Polymer Chemistry, 6*.

de Vlieger, J. J. (2003). Green plastics for food packaging. In *Novel Food Packaging Techniques*, R. Ahvenainen (Ed.), (pp. 519-534): Woodhead Publishing.

Emadian, S. M., Onay, T. T., & Demirel, B. (2017). Biodegradation of bioplastics in natural environments. *Waste Management, 59*, 526-536.

Fang, H.-W., Li, K.-Y., Su, T.-L., Yang, T. C.-K., Chang, J.-S., Lin, P.-L., & Chang, W.-C. (2008). Dip coating assisted polylactic acid deposition on steel surface: Film thickness affected by drag force and gravity. *Materials Letters, 62*(21), 3739-3741.

Gere, D., & Czigany, T. (2020). Future trends of plastic bottle recycling: Compatibilization of PET and PLA. *Polymer Testing, 81*, 106160.

Ghosh, S. K., Pal, S., & Ray, S. (2013). Study of microbes having potentiality for biodegradation of plastics. *Environmental Science and Pollution Research, 20*(7), 4339-4355.

Hossain, K. M. Z., Felfel, R., Ogbilikana, P., Thakker, D., Grant, D., Scotchford, C., & Ahmed, I. (2018). Single solvent-based film casting method for the production of porous polymer films. *Macromolecular Materials and Engineering, 303*, 1700628.

Hu, Y., Daoud, W. A., Cheuk, K. K. L., & Lin, C. S. K. (2016). Newly developed techniques on polycondensation, ring-opening polymerization and polymer Modification: Focus on poly(lactic acid). *Materials (Basel), 9*(3).

Kim, M. Y., Kim, C., Moon, J., Heo, J., Jung, S. P., & Kim, J. R. (2017). Polymer film-based screening and isolation of polylactic acid (PLA)-degrading microorganisms. *Journal of Microbiology and Biotechnology, 27*(2), 342-349.

Land Development Department. Chemical soil analysis process Retrieved November 7, 2019, from http://www.ldd.go.th/PMQA/2553/Manual/OSD-03.pdf

Luyt, A. S., & Malik, S. S. (2019). Can Biodegradable Plastics Solve Plastic Solid Waste Accumulation? In *Plastics to Energy*, Al-Salem, S. M. (Ed.), (pp. 403-423): William Andrew Publishing.

Qi, X., Ren, Y., & Wang, X. (2017). New advances in the biodegradation of Poly(lactic) acid. *International Biodeterioration & Biodegradation, 117*, 215-223.

Speight, J. G. (2015). Environmental Impact. In *Subsea and Deepwater Oil and Gas Science and Technology*, Speight, J. G. (Ed.), (pp. 257-303). Boston: Gulf Professional Publishing.

Tokiwa, Y., & Calabia, B. (2006). Biodegradability and biodegradation of poly(lactide). *Applied microbiology and biotechnology, 72*, 244-251.

Tosin, M., Pischedda, A., & Degli-Innocenti, F. (2019). Biodegradation kinetics in soil of a multi-constituent biodegradable plastic. *Polymer Degradation and Stability, 166*, 213-218.

Viera, J. S. C., Marques, M. R. C., Nazareth, M. C., Jimenez, P. C., & Castro, Í. B. (2020). On replacing single-use plastic with so-called biodegradable ones: The case with straws. *Environmental Science & Policy, 106*, 177-181.

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