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นางสาวธนวรรณ ชลสุวรรณ

**Disintegration test of poly(lactic acid) and some of its based polymer buried in soil**

**Tanawan Chonsuwan**

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in soil

**Student:** Tanawan Chonsuwan          **Student ID:** 5933321123

**Department:** Environmental science

**Advisor:** Associate Professor Roongkan Nuisin, Ph.D

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

…………………………….          Head of Department

(Professor Wanida Jinsart, Ph.D)

SENIOR PROJECT COMMITTEE

…………………………….          Chairman

(Supawin Watcharamul, Ph.D)

…………………………….          Committee

(Sermpong Sairiam, Ph.D)

…………………………….          Project Advisor

(Associate Professor Roongkan Nuisin, Ph.D)

**ชื่อโครงการ:** การทดสอบการสลายตัวของพอลิแลกติกแอซิดและพอลิเมอร์ฐานโดยวิธีการฝังดิน

**นิสิต:** นางสาวธนวรรณ ชลสุวรรณ

**สังกัด:** ภาควิชาวิทยาศาสตร์สิ่งแวดล้อม

**อาจารย์ที่ปรึกษา:** รองศาสตราจารย์ ดร. รุ่งกานต์ นุ้ยสินธุ์

## บทคัดย่อ

ทดสอบการย่อยสลายทางชีวภาพของพอลิแลกติกแอซิด และเซลลูโลส ที่เคลือบพอลิแลกติกแอซิด (PLA) ด้วยวิธีการจุ่มเคลือบที่ระยะเวลา 10, 20 และ 40 นาที ด้วยวิธีการฝังดิน ทดลองโดยทำการฝังดินในสภาวะมีออกซิเจน ระยะเวลาในการทดลอง 45 วัน ที่อุณหภูมิห้อง ติดตามกระบวนการโดยการสังเกต การเปลี่ยนแปลงสัณฐาน การเปลี่ยนแปลงความหนา น้ำหนักที่หายไปและการเปลี่ยนวิเคราะห์การเปลี่ยนแปลงหมู่ฟังก์ชันทางเคมี ผลการศึกษาพบว่า เมื่อนำตัวอย่างมาศึกษาการเปลี่ยนแปลงสัณฐานวิทยา พบว่าเกิดจุดสีดำบริเวณผิวหน้าของกระดาษกรองและกระดาษกรองที่ผ่านการเคลือบด้วยพอลิแลกติกแอซิดเป็นระยะเวลา 10 นาที นอกจากนี้ยังพบจุดสีขาวบริเวณผิวหน้าของพอลิแลกติกแอซิด การศึกษาลักษณะทางกายภาพพบว่า กระดาษกรองมีการเปลี่ยนแปลงความหนามากสุด ส่วนกระดาษที่เคลือบด้วย พอลิแลกติกแอซิด มีการเปลี่ยนแปลงความหนาจากมากไปน้อย คือ กระดาษที่เคลือบด้วย PLA ที่ระยะเวลา 10, 20 และ 40 นาที ตามลำดับ นอกจากนี้การทดสอบพฤติกรรมการบวมตัวของตัวอย่างให้ผลเช่นเดียวกับการเปลี่ยนแปลงความหนาคือ ร้อยละการเคลือบของพอลิแลกติกแอซิดที่เพิ่มขึ้น ส่งผลให้พฤติกรรมการบวมตัวลดลง การทดสอบการเปลี่ยนแปลงของน้ำหนัก ร้อยละน้ำหนักที่หายไปพบว่าเพิ่มขึ้นจากค่าเริ่มต้น โดยกระดาษกรองเพิ่มขึ้นมากที่สุด การวิเคราะห์การเปลี่ยนแปลงหมู่ฟังก์ชั่นด้วยวิธี FTIR พบหมู่ฟังก์ชันที่สำคัญของพอลิแลกติกแอซิด คือ ที่เลขคลื่น 2996 $cm^{-1}$ (C-H), 1748 $cm^{-1}$ (C=O) และเลขคลื่น 1080 $cm^{-1}$ (C–O) กระดาษที่เคลือบด้วยพอลิแลกติกแอซิดที่ระยะเวลา 40 นาที พบว่ามีกลุ่มฟังก์ชั่นที่สำคัญเช่นเดียวกับพอลิแลคติกแอซิด เมื่อเวลาผ่านไป 45 วันพบว่าตัวอย่างทั้ง 2 ชนิดไม่เกิดการเปลี่ยนแปลงของหมู่ฟังก์ชั่น

**คำสำคัญ:** การย่อยสลายทางชีวภาพ, เซลลูโลส, โพลิแลคติกแอซิด, การจุ่มเคลือบ

**Project title:** Disintegration test of Poly(lactic acid) and some of its based polymer buried in soil

**Student:** Tanawan Chonsuwan

**Department:** Environmental Science

**Advisor:** Associate Professor Roongkan Nuisin, Ph.D

## Abstract

Biodegradation test of poly(lactic acid) and cellulose coated with poly(lactic acid) (PLA) by dip-coating technique at the duration of 10, 20 and 40 minutes by burial in the soil. Experimental was tested by buried in soil in at aerobic condition in 45 day at room temperature. Following the experimental by visual observation, changing of thickness, weight loss technique and analytical of changing of functional groups. The study of morphology of sample found that the black spots on the surface of filter paper and filter paper coated with poly(lactic acid) for 10 minutes. The study of physical characteristics found that the filter paper had the highest change in thickness. The most changing of thickness of the paper coated with PLA is the paper coated with poly(lactic acid) at the duration of 10, 20 and 40 minutes, respectively. Swelling behavior of the sample gave the similar trend as the changing of thickness. The higher percentage of coating poly(lactic acid), resulting in decreased swelling behavior. The weight loss of filter paper is highest. FTIR showed that the characteristics functional groups of PLA in form of wavenumber 2996 $cm^{-1}$ (C-H), 1748 $cm^{-1}$ (C=O) and 1080 $cm^{-1}$ (C–O). Functional group analysis of paper coated with poly(lactic acid) at 40 minutes of dip-coating time found that the functional group of samples same as poly(lactic acid). After 45 days, both types of samples did not change the functional groups. Due to samples were tested in a short time. Therefore, the functional groups were not changed.

**Keyword:** Biodegradation, Cellulose, Poly(lactic acid)

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Tanawan Chonsuwan

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## ABBREVIATION

| | | |
|---|---|---|
| ºC | = | degree celcius |
| μm | = | micrometer |
| cm | = | centimeter |
| $cm^{-1}$ | = | percentimeter |
| $cm^2$ | = | square centimeter |
| $CO_2$ | = | carbon dioxide |
| FTIR | = | fourier transmission |
| | | infrared spectroscopy |
| g | = | gram |
| min | = | minute |
| ml | = | milliliter |
| mm | = | millimeter |
| $O_2$ | = | oxygen |
| PLA | = | poly(lactic acid) |
| PP | = | polypropylene |
| w/v | = | weight/volume |
| WC | = | water content |
| WHC | = | water holding capacity |

# CHAPTER I

# INTRODUCTION

## 1.1 Background

The global consumption of petroleum-based plastic has steadily increased. There are several types of synthetic plastics including polyethylene, polypropylene (PP), nylon, polyester, polytetrafluoroethylene, and epoxy etc (Chuensangjun *et al*., 2013; Chae and An, 2018; Nagalakshmaiah *et al*., 2019). The disposal of the non-degradable plastics emerged as a potential harmful to environment. The residues of non-degradable plastics waste were led to the serious pollution in soil and terrestrial ecosystems and directly affected on human health problem (Lui *et al*., 2020). There are many attempts to solve this problem such as the campaign to reduce the use of plastic and recycling process. The development of the bioplastics would lead to an increase in the environmentally friendly of their products. The biodegradable polymers as called "bio-based polymer" can be decomposed by the action of living organisms (Chuensangjun *et al*., 2013; Köhler-Hammer, Knippers, & Hammer, 2016). Bio-based polymers are mainly obtained from renewable raw materials such as starch, vegetable oils, or cellulose (Köhler-Hammer, Knippers, & Hammer, 2016). This material can be degradable in soil environment, through biodegradation process. Biodegradation is defined the process in which microorganisms in the soil such as bacteria and fungi metabolize and break down the structure of polymers (Briassoulis & Mistriotis, 2018). Under aerobic condition, the complete mineralization of polymers is converted to $CO_2$, $H_2O$ and short-chain organic components. The biodegradation reaction of plastics can be described in equation (1.1) (Chinaglia *et al*., 2018);

$$C_{polymer} + O_2 \quad \rightarrow \quad CO_2 + H_2O \qquad\qquad (1.1)$$

Cellulose, the most abundant biopolymer on earth, is an essential structural component of the primary green plants cell wall, forms of algae and the oomycetes (Dhyani & Bhaskar, 2019). Cellulose can be identified as a linear chain of polysaccharide, a homopolysaccharide consisting of linear chain of β (1→4) linked D-glucose glycosidic bond as shown in Figure 1.1 (Prasad *et al*., 2019; Igarashi *et al*., 2012). The cellulose chains are grouped to the cellulose fiber which are linked together by hydrogen bond and van der Waals bonds that is attractive force between three hydroxyl groups including pyranose rings forms intra- and intermolecular hydrogen bonds. However, long- and linear molecules of cellulose does not dissolve readily in water due to a complex molecular structure (Dhyani & Bhaskar, 2019). Cellulose is a bio-based polymer which is commonly used as a single-used packaging for food due to it safe for health of consumer. Cellulose is commonly degraded by group of enzyme cellulase, which can be produced by microorganisms such as bacteria (Prasad *et al*., 2019). Cellulose can be degrading rapidly resulted to non-toxic residue to the environment. However, the cellulose packaging has some disadvantages e.g. cellulose-based paper unable to prevent the moisture and it is also less strong and not resistant to tearing. Accordingly, the bio-based polymer which has the water-proof property is used to modify the packaging of cellulose.
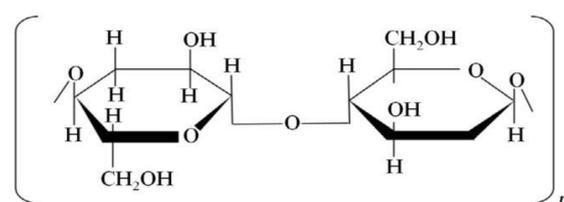


**Figure 1.1** Cellulose structure containing of ß-(1, 4)-glycosidic bonds
(Dhyani & Bhaskar, 2019).

Poly(lactic acid) or PLA is bio-based polymer that currently used to be a coating material due to the hydrophobic property (Rigolin *et al*., 2019). PLA is biodegradable hydrolysable aliphatic semicrystalline. This polymer comes from the direct condensation reaction of its monomer namely lactic acid including L-lactic acid and D-lactic acid. PLA can be easily produced from ring- opening polymerization of

the cyclic lactide dimer (Mehta, Kumar, Bhunia, & Upadhyay, 2005; Karkri, 2017). The international standard testing method (ISO 17566) showed the factors influencing of PLA degradation consist of including water content, pH, temperature (Briassoulis & Mistriotis, 2014), microorganisms, (Karamanlioglu and Robson, 2013) and soil texture (Briassoulis & Mistriotis, 2018). Disintegration of PLA films in soil has been reported (Chuensangjun *et al*., 2013). The different blends of PLA films gave different degradable behaviors which are investigated by Fourier Transmission Infrared spectroscopy (FTIR), weight loss methods and visual observation. Besides, the experimental has shown the other factors are humidity and burial time which effect on biodegradation behavior (Chuensangjun *et al*., 2013). In addition, Karamanlioglu and Robson, (2013) showed the results of influencing of PLA degradation. PLA was buried for 12 months in soil, disintegration and greater weight loss at temperatures of $\geq 37ºC$ and that weight loss is less at lower temperatures. Therefore, the increasing of degradation as the temperature increases indicating that temperature is a key parameter for PLA degradation in humid conditions (Karamanlioglu and Robson, 2013).The life cycle of PLA biodegradation was shown in Figure 1.2. The enzymatic biodegradation was involved the biodegradation process of PLA in soil. The mineralization products of PLA are carbon dioxide $(CO_2)$ and glucose (Martin *et al*., 2014). There are several factors influencing testing of biodegradation in soil such as soil texture, soil water content (WC) that is measured by percentage of water holding capacity (WHC) of soil, the ratio of total organic carbon, total nitrogen, soil pH, and temperature (Briassoulis & Mistriotis, 2018). The optimal temperature in aerobic biodegradation is 40°C (ASTM D5988-12, 2012).
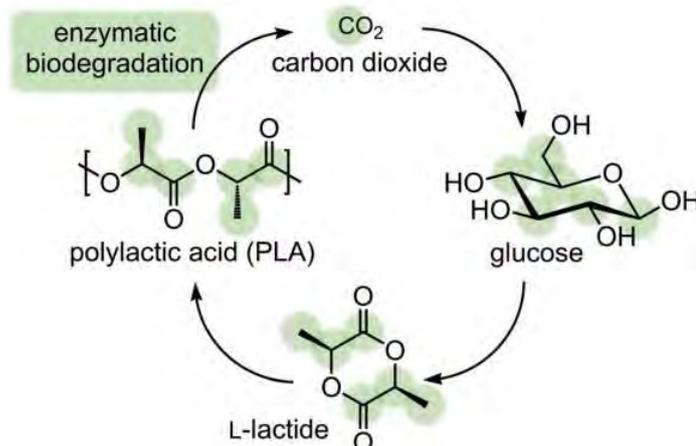
**Figure 1.2** Life cycle of poly (lactic acid) biodegradation (Martin *et al*., 2014)

In this experiment will be focuses on biodegradation of PLA-coated/filter paper in soil. PLA-coated/filter paper will be prepared by dip coating technique. The filter paper will be dip into the PLA solution with various times. The disintegration of PLA-coated paper will be investigated. The PLA-coated/filter paper will be buried in soil under aerobic condition within 45 days of experiment. The disintegration of PLA-coated/filter paper will be characterized. The weight loss of PLA-coated/filter paper will be measured by gravimetric technique. The morphology of PLA will be determined using visual observation. Functional groups of PLA-coated/filter paper will be determined by Transform Infrared Spectroscopy (FTIR) spectroscopy.

## 1.2 Objective

1. To study the disintegration of PLA and coated/filter paper in soil
2. To determine the effects of soil burial laboratory conditions on the disintegration of PLA and coated/filter paper in soil

# CHAPTER II

## THEORETICAL BACKGROUNDS AND LITERATURE REVIEW

This project provides a short overview on the biodegradation of PLA and PLA-coated on cellulose paper. Emphasis is focused mainly on physical change under aerobic biodegradation in soil.

### 2.1 Cellulose

Cellulose is the most abundant biopolymer that mainly component of green plant structure, algae and oomycetes. Cellulose is composed in various parts of the plant which is mainly in cotton up to 90% and 40-50% in wood. The structure of cellulose consisting of D-anhydroglucose ($C_6H_{11}O_5$) unit connected by β-1, 4 glycosidic linkages at C1 and C4 position (Rudin *et al.,* 2013). Cellulose molecules is very hydrophilic, it does not dissolve in water due to a linear chain arrangement. The cellulose chains are grouped from cellulose fiber which linked together by hydrogen bonds and van der Waals bonds forming microfibrils. After that the long chains of microfibrils arranged as a crisscross mesh in a cell wall that provides strength and rigidity to plants that shown in Figure 2.1 (Dhyani & Bhaskar, 2019). Cellulose is commonly degraded by microorganisms. Cellulases is enzyme that hydrolyzed ß-1, 4-glycosidic linkages of the cellulose which can be produced by different microbes such as bacteria (Prasad *et al*., 2019). Degradation of cellulose by bacterial can occur in both aerobic condition and anaerobic condition. The bacterial degradation of cellulose takes place by the group of cellulases that is produced by individual endo and exo-l, 4- ß -glucanases. However, many bacteria lack ß-1, 4- glucosidases (cellobiases). Therefore, they use phosphorylases in order to degrade the cellobiose (Eriksson *et al.,* 1990). Under aerobic condition, the complete mineralization of cellulose is converted to carbon dioxide ($CO_2$), $H_2O$ and short-chain organic components.
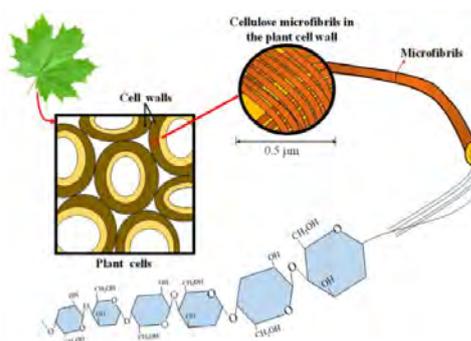
**Figure 2.1** Cellulose fibers in the cell wall (Dhyani & Bhaskar, 2019).

## 2.2 Poly(lactic acid)

Poly (lactic acid) (PLA) is a biodegradable polymer that it will replace the petrochemical plastic (Mehta, Kumar, Bhunia, & Upadhyay, 2005). PLA is used in many applications such as packaging materials, tableware products, and feeding materials for desktop 3D printers. PLA can be produced by fermentation of renewable agricultural produce, such as sugar cane or corn starch (Qin, 2016). An important feature of lactic acid is that it exists in two optically active forms that shown in Figure 2.2. The fermented lactic acid contains 99.5% L-isomer. Production of the cyclic lactide dimer intermediate results in three potential forms. The D- and L-forms are optically active, the D, L- or meso-form is optically inactive. The ratios of all three formats are easily controlled in the process. Ring-opening polymerization of the lactide results in a "family" of polymers with different isomer ratios and in a range of molecular weights. Polymers with high L-levels can be used to produce crystalline products whereas the higher D-levels (15%) result in an amorphous product (Mehta, Kumar, Bhunia, & Upadhyay, 2005)
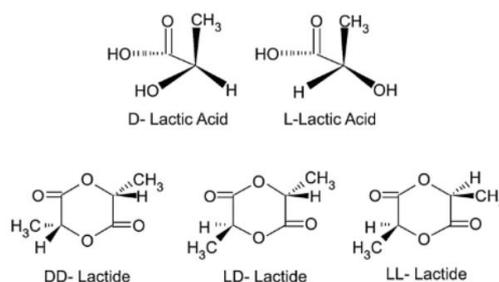


**Figure 2.2** Stereochemistry configurations of lactic acid and lactide molecule (Nofar, M *et al.,* 2019)

### 2.2.1 Poly(lactic acid) synthesis

The process for PLA synthesis from lactic acid was shown in Figure 2.3 (Hu *et al*., 2016). The process is followed by ring-opening polymerization of the cyclic lactide dimer. There are two types of the monomer of polylactic acid, L-lactic acid and D-lactic acid. From both of monomer, there are three possible stereoforms of lactide consist of L-lactide acid, D-lactide and meso-lactide. After formation of oligomer from lactic acid, depolymerization process followed by a ring-opening polymerization of the cyclic lactide dimer (Mehta, Kumar, Bhunia, & Upadhyay, 2005).
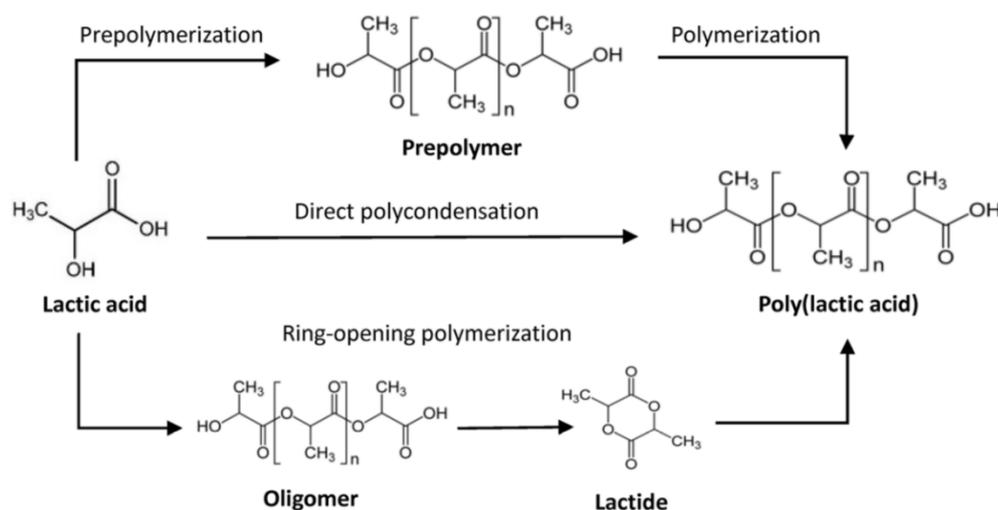


**Figure 2.3** Routes of PLA synthesis from lactic acid monomer (Hu *et al*., 2016)

### 2.2.2 Properties of PLA

The chemical formula of PLA is $(C_3H_6O_3)_n$, and its classification as thermoplastic polymer (Qin, 2016). Thermal, mechanical, and biodegradation characteristics of lactic acid polymers depending on the distribution of stereoisomers within the polymer chains: high- purity of L and D – lactide form stereo regular isotactic poly (L-lactide) and poly (D-lactide), respectively. This is a highly crystalline polymer resulted in a melting point of approximately 180°C and a glass transition temperature in the 55–60°C range. (Mehta, Kumar, Bhunia, & Upadhyay, 2005). PLA is not suitable for high temperature environments (Qin, 2016).

### 2.2.3 Degradation of poly(lactic acid)

The degradation of poly (lactic acid) has been studied (Table 2.1). PLA were degraded in the soil through the action of soil microbial under various factors. Factors influencing the biodegradation behavior of PLA including water content, pH, temperature (Briassoulis & Mistriotis, 2014), microorganisms, time (Karamanlioglu and Robson, 2013) and soil texture (Briassoulis & Mistriotis, 2018). When poly (lactic acid) biodegradable plastic materials are released into the environment, complete biodegradation will take about 2 weeks in the case of dumping into the wastewater treatment system and about 2 months in case of dumping into soil or water environment that shown in Figure 2.4 (Qin, 2016). Biodegradation characteristics of PLA in compost have been studied and reported. The biodegradation of PLA in soil conditions is depends on a temperature and humidity (Luo *et al.,* 2019). Standard testing method (ISO 17566) shows the optimal environmental condition in soil, the optimal water content is 40-60% of the total water holding capacity of the soil and soil pH should be 6-8. ASTM D 5988 shows the carbon-to-nitrogen ratio (C/N ratio or C: N ratio) is adjusted to be between 10:1 and 20:1 by weight to carbon added in the test specimens (Briassoulis & Mistriotis, 2014).

Biodegradation mechanism of PLA that is most accepted involve a two-step degradation process. Initially, the heat and moisture in soil will attack the PLA chain and separated them; therefore, producing low molecular weight polymers and finally it become lactic acid. After that, the microbes in soil will make the pieces of oligomers and lactic acid into methane ($CH_4$) and $CO_2$ under anaerobic and aerobic conditions, respectively (Luo *et al.,* 2019).



**Figure 2.4** Biodegradation of a poly (lactic acid) bottle (Qin, 2016).

**Table 2.1** Literature reviews

| Samples | Methods | Results | Authors |
|---|---|---|---|
| Poly (lactic acid) blends | - Different ratio of PLA blends.<br>- Samples were cut in 2x2 cm$^2$, placed in to plastic net and buried in soil at 5 cm deep for 14 day at room temperature<br>- After the burial, sample were analyzed by weight loss, FTIR and DSC methods. | The PLA blends film that produced from lipase-catalyst showed that it can be degraded greater than pure PLA. | (Chuensangjun *et al.*, 2013) |
| Poly(lactic acid) (PLA) 2003D | - Samples were cut (7×3×0.02 cm and 2×2×0.02 cm), buried in soil at 7 cm deep and incubated at different temperature for 12 months.<br>- Different temperature (25ºC, 37ºC, 45ºC, 50ºC and 55ºC) and<br>- Different time of soil burial.<br>- Different of soil type (soil and compost) | PLA buried in compost is degraded better than burial in soil at the same temperature, degradation to be faster in microorganism-rich environment than abiotic media at 45ºC and 50 ºC and degradation rates were similar at 25ºC and 37 ºC in biotic and abiotic systems | (Karamanlioglu and Robson, 2013) |

| Samples | Methods | Results | Authors |
|---|---|---|---|
| | - After biodegradation test, samples were analyzed by weight loss, tensile strength and GPC methods. | | |
| Poly(lactic acid) (PLA) 2002D and Tributyl O-acetyl citrate (ATBC) | - The sample size of 3 cm x 3 cm was buried in 2 types of soil. <br> - Different type of soil. The soil water content was of $60 \pm 5\%$ and pH was of 6.5 –7.0 <br> - The test was performed both in normal and sterile soils, during a period of 150 days. After that, samples were analyzed for degradation by microbial growth, weight loss, Mechanical testing, GPC, ATR – FTIR, SEM and Contact angle measurement. | After burial, the weight of the specimens changed slightly and all composites became brittle. <br> - The melting point and crystallization of the specimens decreased. Degradation of normal soil is much advanced than in sterile soil because of the microorganisms' action. | (Vasile *et al.*, 2018) |
| Poly(lactic acid) (PLA) | - Different type of samples. <br> - The specimens were buried in soil | - The hydrophilic starch and wood flour increased the growth of | Lv *et al.*, 2017 |

| Samples | Methods | Results | Authors |
|---|---|---|---|
| 2003D | 15 cm deep for 105 days. After degradation test, samples were analyzed by weight loss method, SEM and XPS. | microorganisms. - The end of biodegradation; PLA has a slight increase in weight loss showed that the PLA has low degradation rates in outdoor soils. As a result of low temperature and un-widely of PLA-degrading microorganism in natural soil. | |
| Poly (lactic acid) and Poly (butylene adipate-co-terephthalate) (PBAT) | - The samples were buried from different areas and different time. - PLA was made into a film and cut to 5 cm x10 cm pieces for test. The specimens were buried deep in soil for 40 cm and watered the soil regularly. The samples were buried for 4 months. | - The characteristic peaks of the sample did not change. - DSC showed that the carbon atom content in the molecular structure had decreased whereas the oxygen content increased. | Weng *et al*., 2013 |

## 2.3 Factors influencing the biodegradation of PLA

In general, degradation of polymer is caused by the breakdown of the main chains or the side chains. Both of chemical and biological degradation mechanisms can be involved in the degradation of PLA. There are many factors that affect the degradation of polymer. There are 4 factors follow; (1) chemical structure and molecular weight of polymer; (2) hydrophobic and hydrophilic properties, including polymer surface factors; (3) crystallinity of structure (Tokiwa & Calabia, 2006) and (4) abiotic factors associated with the environmental conditions [temperature, pH, water content, salinity, oxygen and supply of nutrients] affected to microbial growth (Dharmalingam, 2014).

The degradation of polymers demonstrates in changes of the physical structural such as cracking, discoloration and changes of chemical structure such as molecular weight changes, new functional groups etc. (Shah *et al*., 2008). The most accepted PLA degradation mechanism that occurs in two phases: hydrolysis at high temperatures followed by degradation by microbes. The resulting products are $CO_2$ and $H_2O$ under aerobic conditions (Karamanlioglu and Robson, 2013).Microbes, whether fungi or bacteria, are involved in the biodegradation of polymers. Biodegradation of polymers in soil can occur in different conditions, depending on the type of polymer and the type of microbes, which each type has different growth conditions (Shah *et al*., 2008).

PLA has hydrophobic property and high molecular structure. PLA films are quite resistant to water since they degrade slowly. However, PLA are compostable. Extracellular enzymes are released from microbes in order to cause the structure of PLA to break down and dissolve in water to makes it easier to transport through the cell walls of microbes appropriate metabolic pathways that shown in figure 2.5. Extracellular enzymes work on the surface of polymers because they cannot penetrate the material. That can be defining as a process of surface erosion (Dharmalingam, 2014). During the biodegradation, the polymers are shortened to become its monomers. Then, the monomer is transformed into minerals as possible. In order to the microbes can absorb and degraded within the microbes cells for growth (Shah *et al*., 2008).
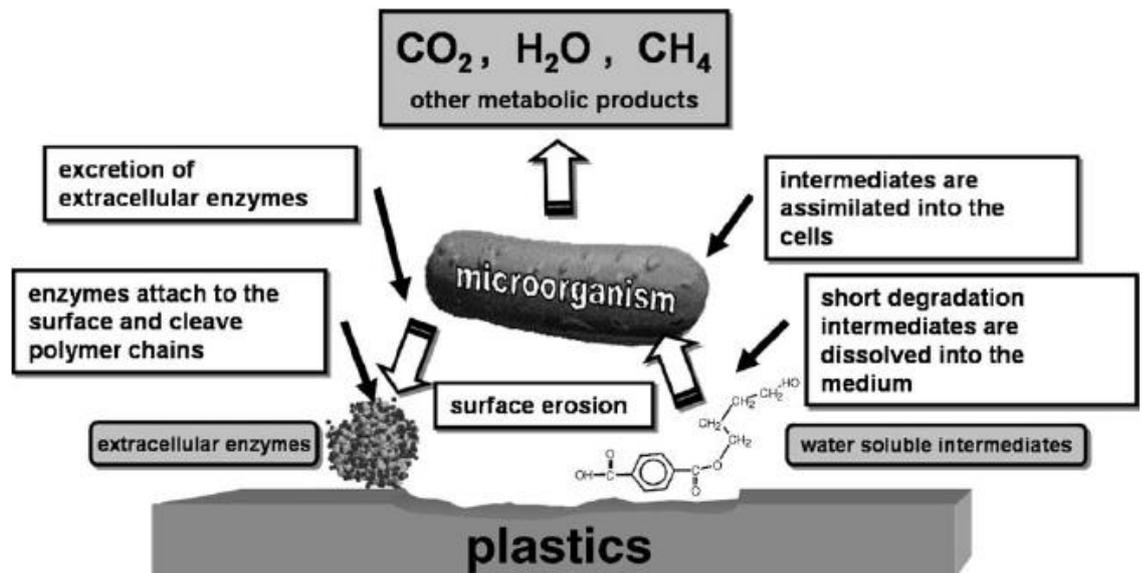
**Figure 2.5** General mechanism of plastic biodegradation (Dharmalingam, 2014).

### 2.4 Standard testing methods (ASTM D5988)

The ASTM committee D20 developed ASTM D 5988-96/2003, "*Standard test method for determining aerobic biodegradation of plastic materials or residual plastic materials after composting in soil*". To test the degradability of product , including compost formulas that claim to cause biodegradation of plastics without inhibiting the growth of microbes. With details as follows;

1.  Soil: soil samples from at least 3 sources are added together. Should be report the source of the soil sample and sieved the soil samples to be less than 2 mm particle size to get rid of rocks and plants.

2.  The source of the inoculum is from solid waste.

3.  In order to check the activity of decomposition in soil, the fully biodegradable materials will be used as reference such as starch or cellulose. If found that CO2 evolution occurs less than 70% within 6 months, a new experiment must be needed with fresh soil and inoculum

4.  The experiment was kept at temperatures between 25 and 27ºC.

5.  Tested in neutral soil.

6. Water holding capacity of the soil used in the test is between 50-70% and the moisture content adjusted by adding distilled water is between 80-100%.

7. The ratio of carbon and nitrogen is adjusted between 10: 1 and 20: 1 (by weight) by adding ammonium phosphate solution.

8. The materials can be used in the test as film, granules, powder, articles and dog bones.

Test of biodegradation of plastic is done in aerobic conditions. Degradation is measured through the occurrence of $CO_2$ as a various time (Dharmalingam, 2014).

### 2.5 Weight loss technique

Biodegradation of fibers or film results in the dissolution partial or complete of material, results in the overall weight of the material to be reduced. The measurement of weight loss is a common method for detecting the biological degradation of various insoluble fiber or film. The measuring of weight loss is one of many methods to use in studies the biodegradation of cellulose fibers in aerobic condition. In addition, measuring weight loss of polymers is also used to follow the biodegradation in anaerobic condition system (Fedorak, 2005). The weight-loss degradability of the film calculated from the weight change of each test piece during the burial in soil. Degradability strongly depended on the specific kind of biodegradable plastic, plastic that has high degradability, the percentage of weight-loss is high (Ohtaki & Nakasaki, 2000). Comparing the ability to weight loss with the hydrolysis which is defined as the molar ratio of carbon loss to $CO_2$ per carbon contained in biodegradable plastics, the amount of $CO_2$ emission from plastic shows the different biological degradability according to the kind of plastics.

# CHAPTER III

# MATERIALS AND METHODS

## 3.1 Research material

### 3.1.1 Chemicals and materials

- Poly(lactic acid) 4020D pellet (commercial grade, NatureWorks®, USA)

- Filter paper (Whatman no.5, Cytiva, USA)
- Polypropylene (PP) film
- Dichloromethane ($CH_2Cl_2$, M.W. = 84.93g/mol, Analytic grade, Mallinckrodt, USA)
- Deionized water

### 3.1.2 Equipment

- Polypropylene boxes
- Vernier caliper (0.05 mm)
- Analytical balance (4 digits) (Mettler Toledo)
- Fourier Transform Infrared Spectrometer (Spectrum One, Perkin-Elmer, USA)

## 3.2 Research Method

### 3.2.1 Soil Characterization

Soil sample was collected from cassava plantation area in Rayong. Soil samples will be determined physical and chemical properties as presented in Table 3.1

**Table 3.1** Methods of experimentation

| Parameter | Methods | References |
| --- | --- | --- |
| Soil texture | Soil sieve method | Briassoulis and Mistriotis, 2018 |
| pH | pH meter | Tetsopgang & Fonyuy, 2019 |
| Soil moisture | Gravimetric | Cole *et al*., 2019 |
| Total Nitrogen | Kjeldahl method | Gómez-Garrido *et al*., 2018 |
| Soil organic matter | Potassium dichromate and sulfuric acid method | Walkley & Black, 1934 |
| Water holding capacity (WHC) | Gravimetric | Mao, Zhang, & Chen, 2019 |

### 3.2.2 Preparation of PLA film

The design amount of PLA4020D pellets (5 %w/v) will be dissolved in dichloromethane. The solution was stirred using magnetic stirrer for 1 hour until clear solution obtained. Dichloromethane was PLA solution was evaporated at 60°C using a hot plate until PLA solution become highly concentrated (approximately 10 %w/v). The concentrated PLA solution (10 %w/v) was cast onto Petri dish for 10 ml and covered lid with design small pinhole to prevent solvent evaporation rapidly. The PLA films was obtained after left in the fume hood at room temperature (Hossain *et al*., 2018). Then, the PLA film was cut into small pieces about 1 cm$^2$. The PLA film was kept for further studies.

### 3.2.3 Preparation of PLA-coated paper by dip coating process

Cellulose filter paper (Whatman 38.5 µm) will be used as the supporter for PLA-coated film. The 60 ml of PLA solution (10% w/v) will be poured into Petri dish. Then, the filter paper was immersed into the solution and covered lid with design small pinhole. The various dipping time of 0, 10, 20 and 40 min was performed. The set-up experiment as shown in Figure 3.1. The PLA-coated/filter paper sample was air-dried to remove the solvent by evaporation (Lin *et al*., 2008). The PLA-coated/filter paper was cut into small stripe of 1 cm$^2$.

PLA Solution

PLA-coated/filter paper
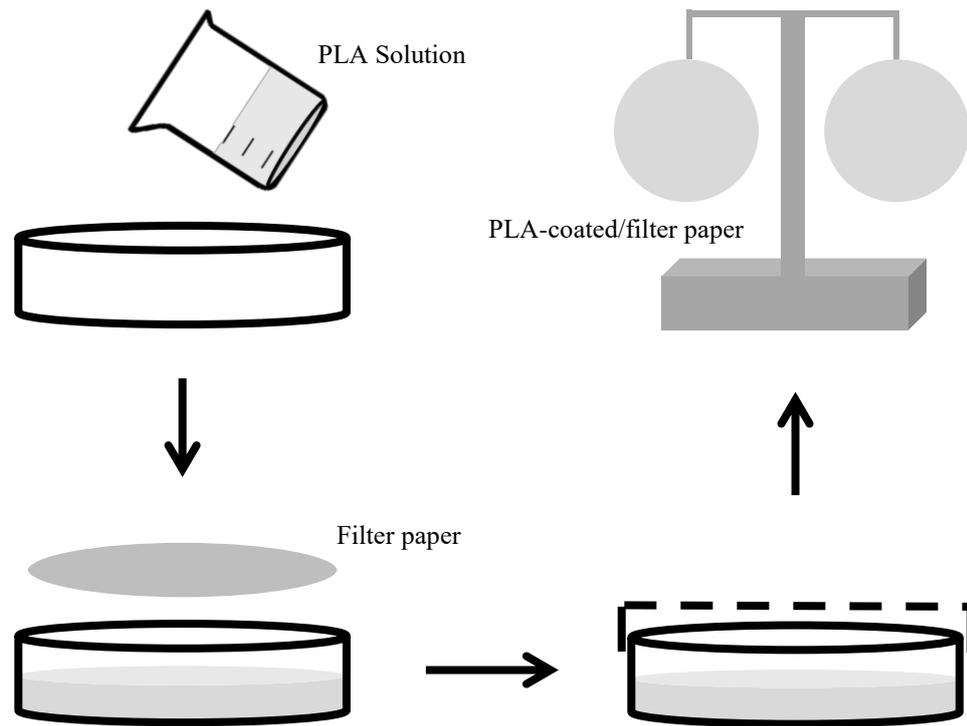
Filter paper

**Figure 3.1** Schematic of PLA-coated/filter paper by dips coating technique

### 3.2.4 Soil burial biodegradation test

The biodegradation was studied under laboratory conditions. The bioreactors of experiment was carried out in polypropylene plastic box and covered with polyethylene thin film. Each bioreactor were contained 20 g of soil sample (< 2 mm). The water content of soil sample was adjusted to 50% of WHC. The bioreactors was kept in the dark at room temperature (25-32°C)(Briassoulis & Mistriotis, 2018). The selected samples of PLA films, filter paper, and polypropylene films was used as references sample. The dip-coated PLA/filter paper with various coating time was studied. Each sample was cut to 1 cm x 1 cm. Five pieces of each samples was inserted in a pockets made of thin non-biodegradable nylon plastics nets. The sample was buried at 2 cm in-depth (Chuensangjun *et al*., 2013). Biodegradation study was carried out under aerobic conditions. The disintegration of sample was investigated in 0, 1, 3, 7, 14, 21, 28, 35 and 45 days.

### 3.2.5 Characterization

#### 3.2.5.1 Physical properties

##### 3.2.5.1.1 Thickness

The thickness of film was measured by Vernier caliper (Ohtaki& Nakasaki, 2000).

##### 3.2.5.1.2 Swelling

The swelling was tested by immersed the samples in distilled water in period of 1,2,3,4 and 5 hours. The percentage of swelling was calculated by equation (3.1) (Lanny Sapei *et al*., 2015)

$$\% \text{ Sweling} = \frac{w - w0}{w0} \text{x100\%} \quad \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots(3.1)$$

where  $W_0$ is the initial weight of sample

W is weight of sample after swelling experimental

##### 3.2.5.1.3 Weight loss

Six types of specimens will be used for experiment (Table 3.1) in order to determine their weight-loss degradability. The percentage of weight loss was calculated based on dry weight. The samples was incubated at 80°C for 24 hour, and put into desiccator for 24 hour. The samples were weighted of sample before and after biodegradation experiment were measured. The percentage of weight loss can be calculated with a weight loss equation (3.2) (Shovitri *et al*., 2017),

$$\% \text{WL} = \frac{wi - wf}{wi} \text{x100\%} \quad \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots(3.2)$$

where, $W_i$ is the initial dry weight before biodegradation test (g)

$W_f$ is the dry weight after biodegradation test (g).

**Table 3.2** Composition of samples for physical properties testing.

| Sample name | Composition | | |
|---|---|---|---|
| | PLA (%) | Cellulose (%) | Dipping time (min) |
| Cellulose paper | 0 | 100 | 0 |
| PP | 0 | 0 | 0 |
| PLA | 100 | 0 | 0 |
| Cellulose-PLA_T10 | 31.6 | 68.4 | 10 |
| Cellulose-PLA_T20 | 32.4 | 67.6 | 20 |
| Cellulose-PLA_T40 | 37.1 | 62.9 | 40 |

**3.2.5.2 Morphology of PLA-based film**

Morphology of PLAbased/paper film samples were observed by visual observation. The digital photograph was recorded.

**3.2.5.3 Fourier Transform Infrared (FTIR) spectroscopy**

Functional groups of PLA-based film was determined using Fourier Transform Infrared Spectro (Spectrum One, Perkin-Elmer, USA)

# CHAPTER IV

## RESULTS AND DISSCUSSION

### 4.1 Soil characterization

Soil sample were collected from cassava plantation area in Rayong on Thursday 7th November 2019. The pH of soil sample was measured at pH 6.83, indicating that the soil is neutral. The chemical properties were determined. The results showed that the organic carbon content equal to 0.5%, organic matter equal to 0.8%, indicating that the low level of organic matter. The soil texture was shown sandy loam (Land Development Department, 2004).

### 4.2 Morphology of PLA-based film

Figure 4.1, the virtual observation of the samples was reviewed. The results showed that PP film did not change their color and shape throughout the time of soil burial study. The structure of PP is composed of long-chain hydrocarbon of propylene monomers. The carbon-carbon bonding (-C-C-) was difficult to degrade by microbes due to its high hydrophobic property (Sable, S. *et al*., 2019). PLA film disintegration occurs only slightly. One can be seen that, there is a change in color. It was found that a small white spot was observed on surface of PLA film at Day 45th Shah *et al*., (2008) reported the occurrence of crack, agglomerates, pits, and/or grooves on the surface of plastic buried in the soil as a result of microbial enzymatic activity. Color of cellulose is changed on the 45th day, sample become light yellow. Changing the morphology of cellulose coated with PLA at different times showed that the shape and color of all types of coated paper unchanged. When observing the surface of all 3 types of samples, it was only found that the he black spots on the surface of cellulose-PLA_T10. While, the other two types has unchanged. Expected as a result of Cellulose-PLA_T10 has a thin layer of PLA coated.

|  | DAY 0 | DAY 7 | DAY 21 | DAY 35 | DAY 45 |
|---|---|---|---|---|---|
| **PP** | | | | | |
| **Cellulose** | | | | | |
| **PLA** | | | | | |
| **Cellulose-PLA_T10** | | | | | |
| **Cellulose-PLA_T20** | | | | | |
| **Cellulose-PLA_T40** | | | | | |
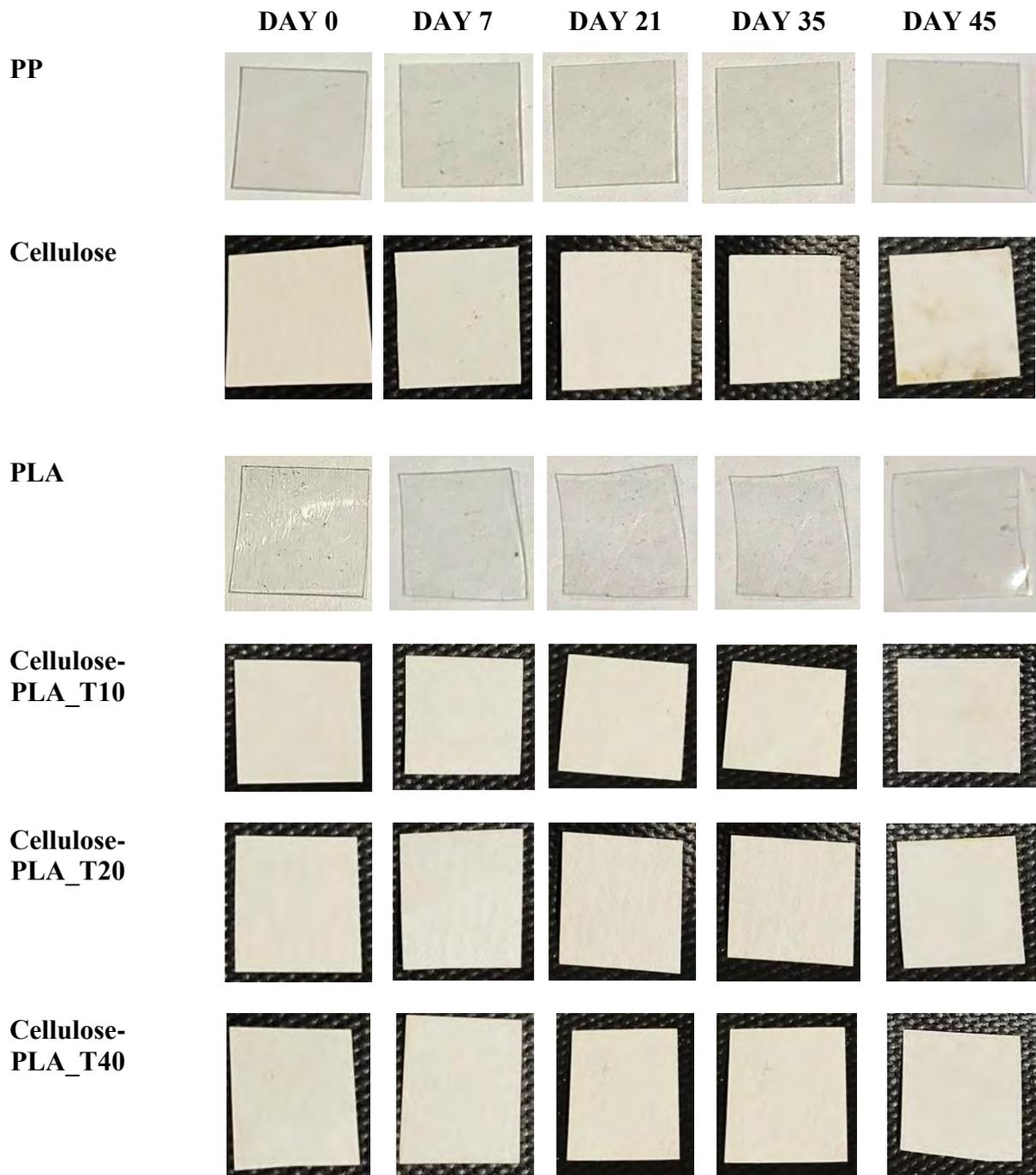
**Figure 4.1** Changing of samples during biodegradation in soil burial experiment; PP, PAPER, PLA, Cellulose-PLA_T10, Cellulose-PLA_T20 and Cellulose-PLA_T40

### 4.3 Physical properties of film

#### 4.3.1 Thickness

The film thickness of PLA, cellulose/PLA-coated was determined, as shown in Figure 4.1. After the burial in soil, the thickness of each type of sample is increased except polypropylene (PP). The thickness of PP film was unchanged at all times of soil burial. Since PP is a linear hydrocarbon polymer which contain -C-C- bonding, it is not biodegradable, PP has hydrophobic properties and does not contain the functional group, swelling did not occurred (Sable *et al*., 2019). While the thickness of PLA film was changed, the initial thickness of $0.07 \pm 27.4$ mm and the thickness gradually increased. After 45 days, the thickness increased equal to $90 \pm 22.4$ mm or 28.6% from initial. The highest thickness change was found in cellulose sample. The initial thickness was 0.15 mm and the thickness increased steadily until the $7^{th}$ day of burial. The thickness of the specimen was constant with a thickness of 0.2 mm or 33.3% and constant until Day $45^{th}$. Cellulose is porous that allowing water and air to infiltrate between fibers. As a result, the thickness of the filter paper is increasing from the initial (Alexandersson & Ristinmaa, 2019).

Testing the change of cellulose/PLA-coated, found that the cellulose-PLA_T10 had the highest thickness changes. The initial thickness of 0.2 mm and the thickness gradually increased until the $7^{th}$ day of burial. The thickness of the sample was found stable, with a thickness of $0.23 \pm 27.4$ mm or 15%, and the thickness was increased after day 45 equal $0.24 \pm 22.4$ mm or 20%, while the thickness of the cellulose-PLA_T20 has an initial thickness of $0.17 \pm 27.4$ mm. The $3^{rd}$ day of burial, the thickness of the sample increased to 0.20 mm and remained stable. After 45 days the thickness of cellulose-PLA_T20 increased to $0.24 \pm 25$ mm or 25%, which the thickness of the sample increased less than the thickness of cellulose-PLA_T10. The cellulose-PLA_T40 has an initial thickness of $0.20 \pm 0$ mm and there is unchanged in thickness. Until the $45^{th}$ day the thickness was increased to $0.23 \pm 27.4$ mm or 15%. The specimens rarely changed the thickness.

#### 4.3.2 Swelling

All 4 types of samples were studied for swelling behavior: PLA, Cellulose-PLA_T10, Cellulose-PLA_T20 and Cellulose-PLA_T40. It was found that different percentage of PLA coatings that affected the swelling behavior of the samples. The

high percentage of PLA coating (sample Cellulose-PLA_T40). The swelling behavior tends to be similar to PLA. While, the samples with less percentage of PLA coating, the swelling behavior of samples was similar to cellulose. These may cause that Cellulose-PLA_T40 has a thick coating As a result, the sample has high hydrophobic property (S. N, *et al*., 2020). The time of soaking increased, all specimens are trend to more swelling, except PLA. The study of the swelling of PLA, may take longer time to experiment.

### 4.3.3 Weight loss

The weight losses of each sample were determined, as shown in Figure 4.3. Each type of sample has different weight changes profile. PP film has an initial weight of $0.0142 \pm 0.0005$ g and at Day $45^{th}$, the weight is decreased of 0.1%. Expected to the degradation occurs very little or no degradation Sable, *et al*., (2019) reported on the results of PP film biodegradation testing using gravimetric weight loss method. Jeyakumar *et al*., (2013) shows that PP film has very little weight change as well. The change in the weight of the PLA film showed that the weight after burial was reduced to a similar proportion, except for the $28^{th}$ day, weight of samples was increased from the initial weight (-3.8%). It was found that the weight change of cellulose occurs very rarely, with the initial weight of $0.0106 \pm 0.0009$ g. After 45 days of study have passed, the percentage of weight loss of cellulose is -19.9%, shown that the weight of the sample increases from the initial weight, expected that the result of specimens after burial was not dried. Therefore, moisture inside the sample may remain, resulting in the weight of the sample increasing from the initial. The test for weight changing of cellulose/PLA-coated film, found that all 3 types of samples gave similar results, the weight of each type of samples increased from the initial weight. When the cellulose-PLA_T10 was buried, the sample weight increased from the initial weight in every period. With initial weight of $0.0164 \pm 0.001$ g, after 45 days the percentage of weight loss to -0.6%. The initial weight of cellulose-PLA_T20 was $0.0177 \pm 0.002$ g, % weight loss after 45 days is -1.3% and the initial weight of T40 was $0.02 \pm 0.001$ g, percent-weight loss after 45 days is -0.8%. The longer time of film coating will result in percentage is increased and slightly increasing of film thickness (Soares *et al*., 2016) but does not affect % to weight loss.

**Figure 4.2** Changing of thickness of degradation time in soil burial; PP, Cellulose, PLA, Cellulose-PLA_T10, Cellulose-PLA_T20 and Cellulose-PLA_T40
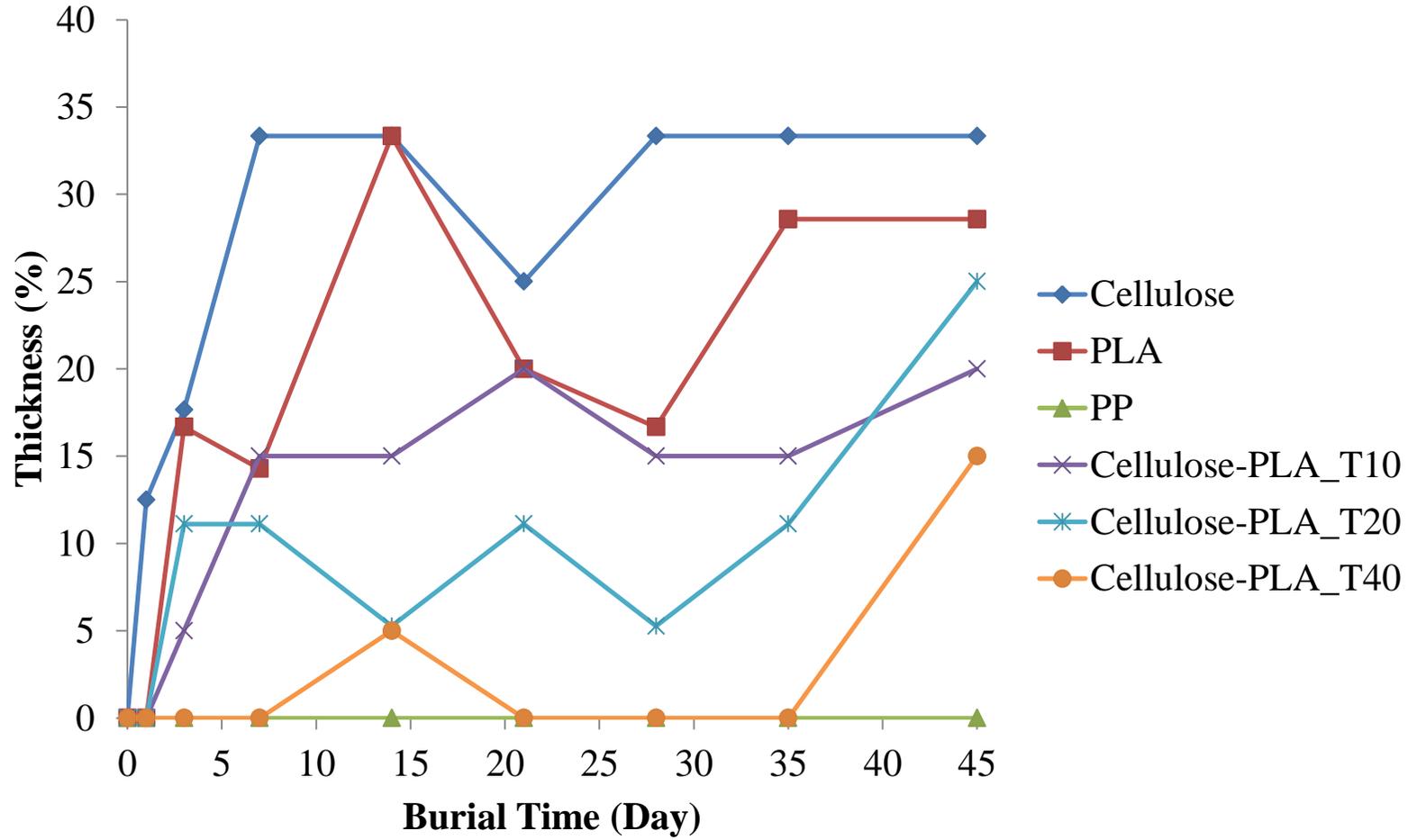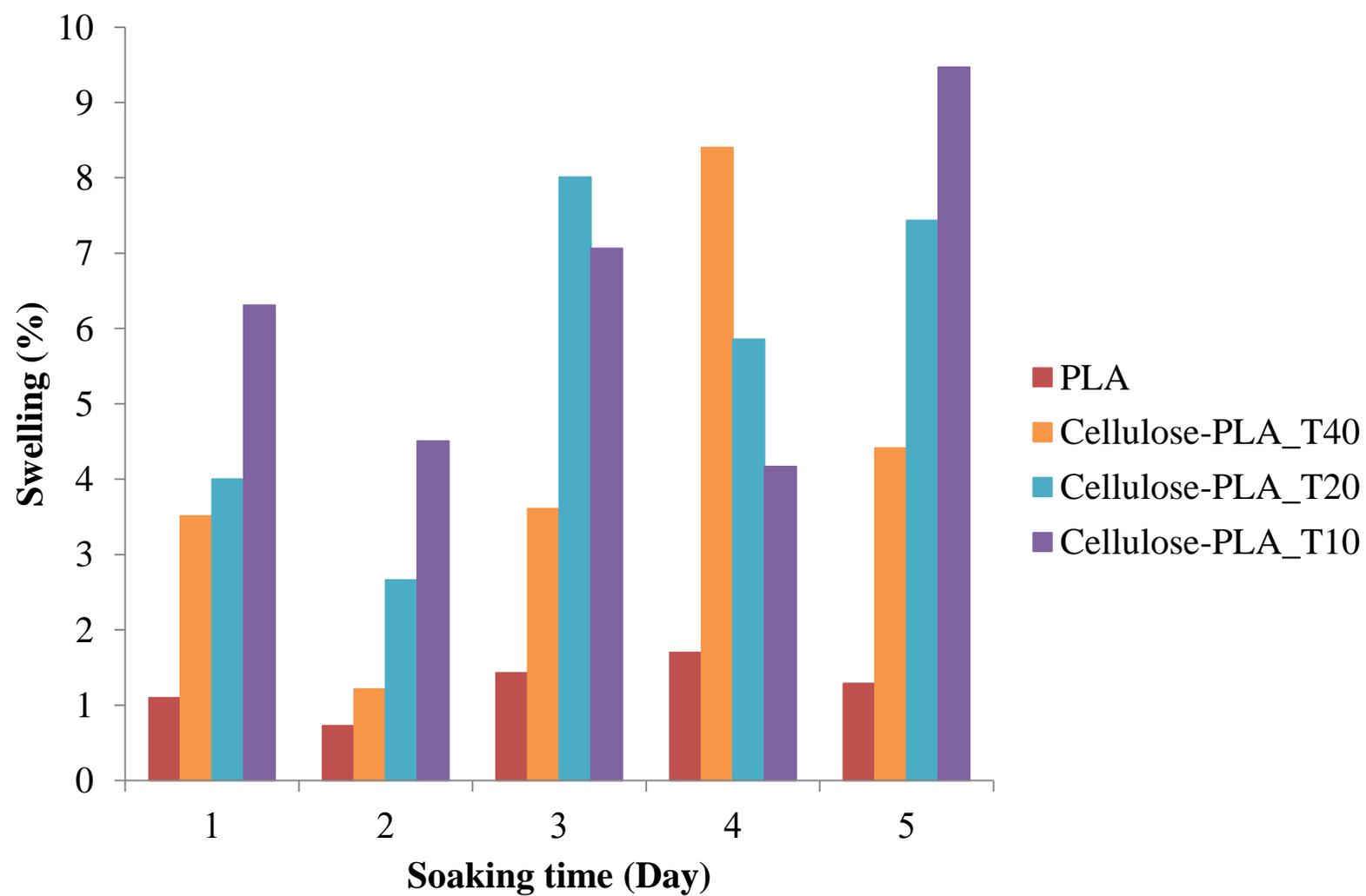
**Figure 4.3** Swelling behavior of samples; PLA, Cellulose-PLA_T10, Cellulose-PLA_T20 and Cellulose-PLA_T40
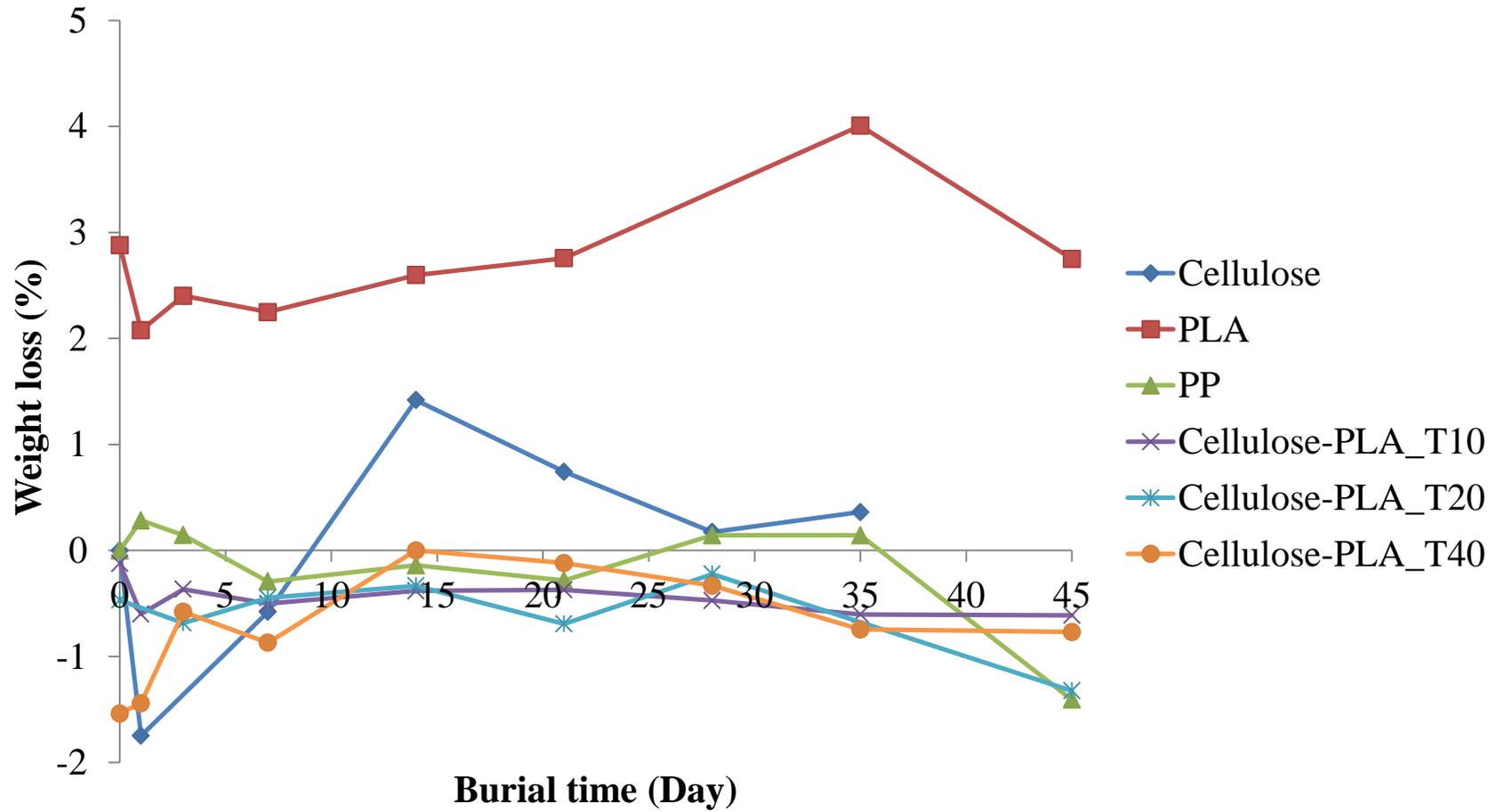
**Figure 4.4** Weight loss of degradation time in soil burial; PP, Cellulose, PLA, Cellulose-PLA_T10, Cellulose-PLA_T20 and Cellulose-PLA_T40

**4.4 Characterization of PLA, cellulose and cellulose-PLA_T40**

The chemical structure of filter paper (cellulose), PLA film, and cellulose-PLA_T40 was analyzed by ATR-FTIR technique. The results were shown in Figure 7 and Table 4.1. Spectrum of cellulose shows the hydroxyl group at the absorption band at 3334.98 $cm^{-1}$ and represented a broad C-H hydroxyl groups symmetric peak at 2899.22 $cm^{-1}$ and C = O the ester carbonyl group at 1635.12 $cm^{-1}$. The absorption band at 1314.95 and 1029.56 $cm^{-1}$ is characteristic of the C-H group symmetric and C-O group of secondary alcohols respectively. A band at 896.67 cm−1 is assigned to β-glycosidic linkage between glucose units. Comparison of Cellulose_DAY45 with the spectrum of Cellulose_DAY0, there is no change in the function groups. Meanwhile, the ATR-FTIR characterization cannot describe the change of functional groups of sample within short period of soil burial studies. The specimens did not biodegradation (Chieng, *et al*., 2013).

From FTIR spectrum of the PLA film. The absorption band at 2995.9 $cm^{-1}$ is assigned to C-H hydroxyl groups asymmetric stretching. The band at 2946.15 $cm^{-1}$ is assigned to C-H hydroxyl groups symmetric stretching. Bending frequencies for C=O the ester carbonyl group and C–O group of carbonyl groups have been identified at 1747.79 and 1080.13 $cm^{-1}$, respectively. Cellulose-PLA_T40 shows almost similar absorption peaks as PLA. Comparison of PLA_DAY45 and cellulose-PLA_T40_DAY45 with the spectrum of PLA_DAY0 and cellulose-PLA_T40_DAY0, there is no change in the functional groups.

**Table 4.1** Attenuated Resonance Fourier transforms infrared (ATR-FTIR) spectra of Cellulose, PLA, and Cellulose- PLA_T40 before and after soil burial degradation**.**

| Samples | Wavenumber (cm$^{-1}$) | Characteristic |
|---|---|---|
| Cellulose_D0 | 3334.98 | Hydroxyl OH stretching |
| | 2899.22 | C-H hydroxyl groups symmetric stretching |
| | 1635.12 | C=O the ester carbonyl group stretching |
| | 1314.95 | C-H group symmetric stretching |
| | 1029.56 | C-O group of secondary alcohols |
| | 896.67 | β-glycosidic linkage |
| PLA_D0 | 2995.9 | C-H hydroxyl groups asymmetric stretching |
| | 2946.15 | C-H hydroxyl groups symmetric stretching |
| | 1747.79 | C=O the ester carbonyl group stretching |
| | 1080.13 | C–O group of carbonyl groups stretching |
| PLA/CEL_T40_D0 | 2995.63 | C-H hydroxyl groups asymmetric stretching |
| | 2946.02 | C-H hydroxyl groups symmetric stretching |
| | 1748.44 | C=O the ester carbonyl group stretching |
| | 1083.42 | C–O group of carbonyl groups stretching |
| PLA/CEL_T40_D28 | 2995.57 | C-H hydroxyl groups asymmetric stretching |
| | 2945.92 | C-H hydroxyl groups symmetric stretching |

| Samples | Wavenumber (cm$^{-1}$) | Characteristic |
|---|---|---|
| | 1748.43 | C=O the ester carbonyl group stretching |
| | 1083.23 | C–O group of carbonyl groups stretching |
| Cellulose_D45 | 3335.15 | Hydroxyl OH stretching |
| | 2898.67 | C-H hydroxyl groups symmetric stretching |
| | 1640.34 | C=O the ester carbonyl group stretching |
| | 1314.95 | C-H group symmetric stretching |
| | 1029.82 | C-O group of secondary alcohols |
| | 897.49 | β-glycosidic linkage |
| PLA_D45 | 2995.92 | C-H hydroxyl groups asymmetric stretching |
| | 2946.09 | C-H hydroxyl groups symmetric stretching |
| | 1747.81 | C=O the ester carbonyl group stretching |
| | 1080.17 | C–O group of carbonyl groups stretching |
| PLA/CEL_T40_D45 | 2995.66 | C-H hydroxyl groups asymmetric stretching |
| | 2945.79 | C-H hydroxyl groups symmetric stretching |
| | 1748.55 | C=O the ester carbonyl group stretching |
| | 1083.24 | C–O group of carbonyl groups stretching |
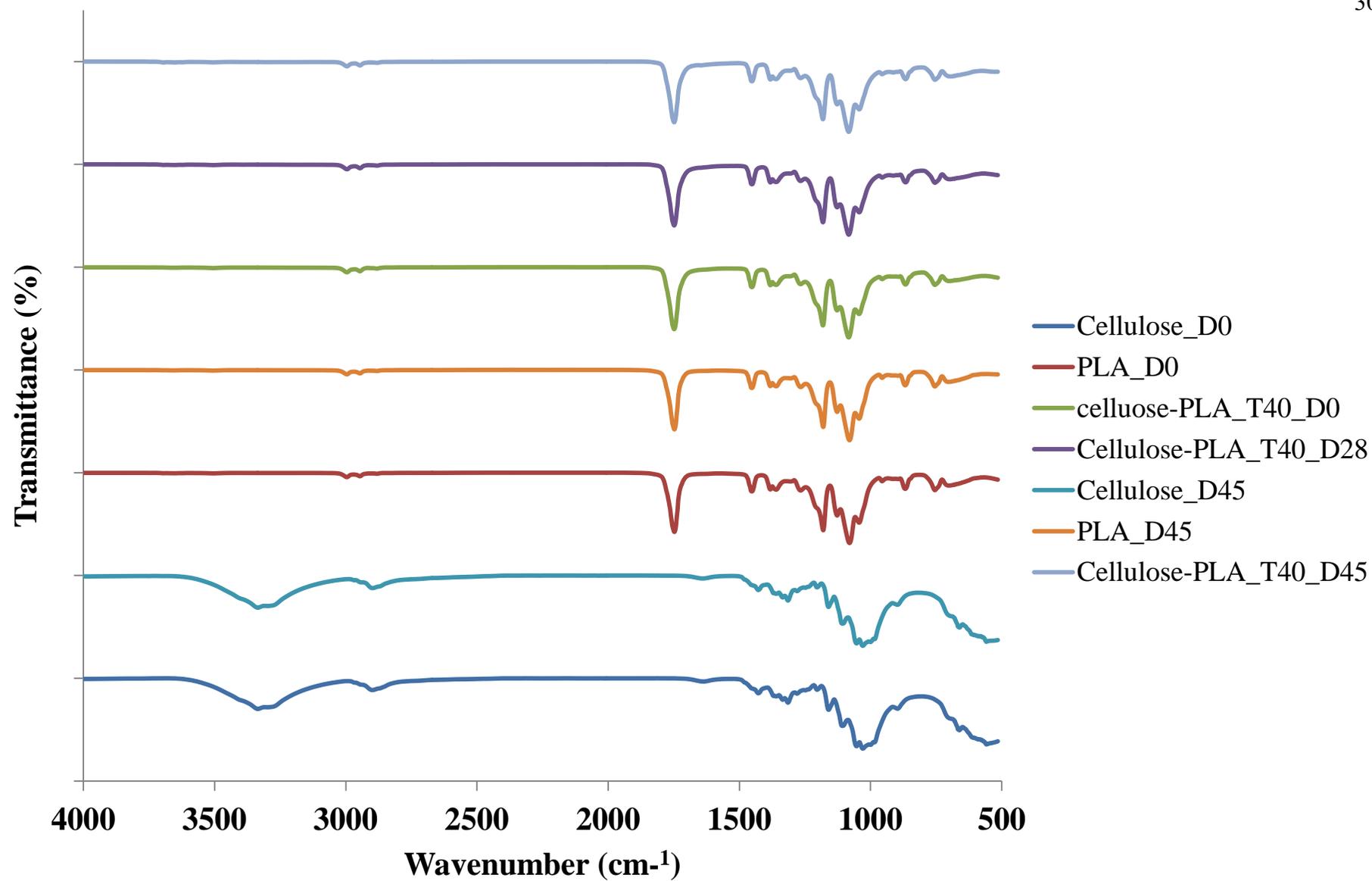
**Figure 4.5** Fourier transform infrared (FTIR) spectra of Cellulose, PLA, and Cellulose- PLA_T40 before and after soil burial degradation

# CHAPTER V

# CONCLUSION

PLA, PP, cellulose and cellulose/PLA- coated films were used for disintegration by buried in soil at room temperature in 45 days. PLA was dissolved in DCM and casted on the Petri dish. Cellulose/PLA-coated were prepared by dip-coating technique. The specimens were used in biodegradation in aerobic conditions. Soil sample was characterized. Soil texture is loamy sand.

The samples were study the morphology by visual observation. It was found that PP, cellulose-PLA_T20 and cellulose-PLA_T10 did not any changes. The PLA was revealed the opaque spots after 45 days of soil burial. While cellulose and cellulose-PLA_T10 is the most change, there is the changing of color from the initial time. Sample changed from white to yellow color and found the black spot on the surface after 45 day.

The study of physical properties, thickness change was ordered of cellulose the thickness increased equal to $90 \pm 22.4$ mm or 28.6% from initial subsequent PLA, cellulose-PLA_T10 and cellulose-PLA_T20. On the other hand, cellulose-PLA_T40 and PP were rarely changed the thickness. The study of swelling behavior, the different percentage of PLA coating of cellulose/PLA- coated film show that the higher percentage of PLA coating affected the percentage of swelling is decreased. The results showed that the percentage of PLA coating is related to the changing of thickness and swelling behavior of samples. The percentage of weight loss study of the specimens. The weights of all samples were changed only slightly, except PLA.

FTIR show that the functional groups of cellulose including hydroxyl, C-H hydroxyl groups symmetric, C=O the ester carbonyl group, C-H group symmetric, C-O group of secondary alcohols and β-glycosidic linkage at 3334.98 cm$^{-1}$, 2899.22 cm$^{-1}$, 1635.12 cm$^{-1}$, 1314.95 cm$^{-1}$, 1029.56 cm$^{-1}$ and 896.67 cm$^{-1}$, respectively. In addition, the

characteristics functional groups of PLA for C-H hydroxyl groups asymmetric, C-H hydroxyl groups symmetric, C=O the ester carbonyl group, C–O group of carbonyl groups at 2995.90 cm$^{-1}$, 2946.15 cm$^{-1}$, 1747.79 cm$^{-1}$ and 1080.13 cm$^{-1}$, respectively.

# BIOGRAPHY

**Name**:          Tanawan Chonsuwan

**Date of birth**:  08 September 1997

**Place of birth**: Trang Province, Thailand

**Address**:        32/86 Moo 2, Bankuan Sub- area Meaung Area, Trang, 92000

**E- mail**:         prangchonsuwan@gmail.com

**Graduation**:   Bachelor of Science (Environmental Science), Chulalongkorn
                 University 2020 from the Faculty of Science