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# Hydrolysis of Polylactic Acid Fiber by Lipase from *Porcine pancreas*

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**Abstract**

This study is to optimize the enzymatic processing conditions of Polylactic Acid (PLA) fiber using lipase from Porcine pancreas as an environmental technology. Hydrolytic activity dependent on pH, temperature, enzyme concentration, and treatment time, and structural change of PLA fiber were evaluated. The PLA fiber hydrolysis by lipase was maximized at 50% (o.w.f) lipase concentration 50°C for 120 minutes under pH 8.5. There was a change of the protein absorbance in the treatment solution before and after the lipase treatment. In addition, there was no substantial change in the molecular and crystalline structures of PLA by lipase treatment as confirmed by DSC, XRD, and FT-IR.

***Key words:*** Polylactic acid, PLA, Fiber, Hydrolysis, Lipase

## I. Introduction

In the 21st century, the textile industry has focused on achieving green technology, so new processing for PLA fibers based on biotechnology and ecotechnology has been required. The applications of green technology in the textile industry can be divided into the following two applications: (1) recycling processing using biomass such as bean fiber, arginine fiber, chitosan fiber, and polylactic acid fiber, and (2) eco-processing. Polylactic acid (PLA) is a linear aliphatic thermoplastic polyester made up of lactic acid (2-hydroxy propionic acid) building blocks <Fig. 1> and can be derived from 100% renewable sources such as corn (Bisswanger, 2004), so it has received considerable attention as a biomass material in the textile industry. It is highly marketable owing to the low unit cost of its raw materials and is available from agricultural renewable resources. It is biodegradable, and its mech anical properties such as density, glass transition temperature, tensile strength, and Young's modulus are similar to those of polylethylene terephthalate (PET) or nylon (Korea Textile Inspection & Testing Institute, 2005; Sawada et al., 2007).

Studies on PLA fibers have focused on replacing PET and polytrimethyl terephthalate (PTT) with biodegradable plastic (Blackburn, 2005; Drumright et al., 2000; Oksman et al., 2003). Owing to their advantages, various types of PLA fibers have been manufactured and developed in nonwoven, fabric, films (Kim, 2005; Sawada et al., 2007). Some of the key performance features that distinguish PLA fibers from other fiber materials in <Table 1> (Tokiwa & Jarerat, 2004). Generally, PLA is aliphatic polyester, thus the moisture regain are 0.6%, it is superior to that of PET. Also, PLA has lower tensile strength than PLA. Furthermore, garments made from 100% PLA feel more comfortable. Therefore, to apply PLA fibers in fields of textile industry, effects of pre-treatments processing such as desizing, alkaline scouring, hydrophilic procesisng for PLA is demanded. For using PLA fibers in the textile industry, a post-processing method should be developed. Also, by post-processing, fiber properties such as moisture regain, wettability could be improved. However, as PLA is linear aliphatic polyester, its chemical resistance to hydrolysis is poor other than synthetic fibers (Farrington et al., 2005). so use of exi-

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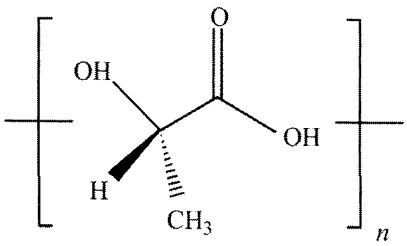



**Fig. 1. Structure of PLA.**

**Table 1. Key performances of PLA fiber**

| |
|---|
| More hydrophilic than PET |
| Lower density than PET |
| Excellent hand, drape and feel |
| Good resilience |
| Excellent crimp and crimp retention |
| Controllable shrinkage |
| Unaffected by UV light |
| Dyeable with disperse dyes |
| Outstanding processability |

sting chemicals in the post-processing on PLA fibers can damage PLA fibers by decreasing their strength, and elasticity; further, the chemicals can contribute to environmental pollution (Reddy et al., 2008). Thus, environment-friendly processing is needed for PLA fiber in order to minimize the damage and maintain the environment-friendly advantage of the fiber. From among several post-processing methods, enzyme processing has been proposed and used to post-processing for natural, synthetic, and recycled fiber (Cavaco-Paulo & Guebitz, 2003; Guebitz & Cavaco-Paulo, 2007; Kim & Song, 2006; Lee et al., 2009; Lee & Song, 2010). Moreover, normally occurs only on the fiber's surface without affecting the fiber's interior structure thus, the fiber's properties can be preserved.

In light of this discussion, we propose enzyme processing as a suitable, environment-friendly processing method for PLA fibers. Hydrolysiable enzymes of ester bond's on PLA fibers are lipases, esterases, and proteases. Lipases (EC. 3.1.1.3) are tryacylglyserole lipase, and known as representative lipolysis enzyme. They can degrade polymer chain of various aliphatic polyesters randomly (Mayumi et al., 2008; Tokiwa & Jarerat, 2004) and accept long-chain fatty acids as substrates in water-soluble system (Buchholz et al., 2005). By enzymatic treatment in a short time, the improvements of properties such as hydrophilic properties of fiber are expected. Therefore, in our study, we used lipase from *Porcine pancreas* for PLA hydro-lysis. In addition, the optimal conditions for the enzyme treatment was determined in terms of the pH, the treatment temperature, time, and the enzyme concentration. Moreover, the hydrolytic effect of enzymes on the structural properties of the fiber were measured by differential scanning calorimetry, X-ray diffraction analysis, infrared spectroscopic analysis and the PLA fiber surface was observed.

Through this study, we intend to present the optimal conditions for enzyme processing using lipase as an environment-friendly processing for PLA fibers.

## II. Experimental Design

### 1. Materials

One hundred percent polylactic acid nonwoven supplied from Toray was used for the experiment and lipase from *Porcine pancreas* (Sigma Chemicals Co., U.S.A) were used (Table 2)-(Table 3). Tris (pKa=8.06, Sigma Chemicals Co., USA) was used as buffers for lipase. 0.1N Sodium hydroxide solution (Junsei Chemicals, Japan), Thymolphthalein (TPH, ACS reagent, Aldrich Chemicals Co., U.S.A) as an indicator, 95% ethanol (Duksan Pure Chemicals, Korea) were used for titration method. Bradford reagent (Sigma Chemicals Co., U.S.A) was used for protein assay. All chemicals were used without further purification.

**Table 2. Characteristics of PLA nonwoven**

| Purity (%) | Thickness (mm) | Weight ($g/m^2$) | Manufacturing Method |
|---|---|---|---|
| 100 | 0.126 | 30 | Spunbond |

**Table 3. Properties of enzyme**

| Enzyme | Source | Activity | Form | Manufacturer |
|---|---|---|---|---|
| Lipase (EC. 3.1.1.3) | Porcine pancreas | 15-35 unit* / mg | Powder | Fluka |

*One unit corresponds to the amount of enzyme which hydrolyzes 1μmol of fatty acid from a triglyceride per minute.

## 2. Methods

### *1) Lipase Treatment*

All lipase treatment for measuring properties of PLA nonwovens was performed in 50mM TRIS buffer solution, using a liquor ratio was 50:1. Each nonwoven sample was cut to a dimension by 20×20cm. PLA nonwovens were treated by lipase at 150rpm using a shaking water bath (BS-21, Jeio Tech., Korea) with optimal lipase treatment conditions. Following treatment, the residual lipase of the samples was inactivated by heating them at 90°C for 10min. After inactivation, the samples were thoroughly washed with water at a liquor ratio of 100:1 and then dried at room temperature.

### *2) Measurement of Hydrolytic Activity*

In this study, in order to measure amount of carboxyl group by hydrolysis of PLA fiber, titration method was used (Kim & Song, 2006; Lee & Song, 2009). Titration method is known for lipase assay. The hydrolytic activity figured out the amount of carboxyl hydrolyzed from ester group and it was presented by NaOH consumption. For this, PLA sample was prepared approximately 0.1g of weight. All specimens are placed into 20mL of bial bottle, and 8mL of buffer solution was added. Enzymatic treatment was performed depending on pH, temperature, treatment time, and lipase concentration; pH from 7.0 to 9.0, temperature from 30°C to 60°C, treatment time from 10 to 300min, and lipase concentration from 1 to 200% (o.w.f). After enzymatic treatment, 20mL of ethanol and 4 drops of 0.9% TPH were added to the solution. In addition, 0.1N sodium hydroxide was added until the solution color turned light blue. We then recorded the volume of 0.1N sodium hydroxide used in the test. Each test was repeated five times.

### *3) Enzyme Protein Absorbance*

The change of enzyme protein absorption was evaluated according to the Bradford assay (Lee et al., 2010), using UV-Vis spectroscopy (M-3000, Scinco Co. Ltd., Korea) compared with before and after lipase treatment.

### *4) Tensile Properties*

The tensile strength of the lipase-treated PLA nonwovens was determined using an Universal Testing Machine (H 100KS, Hounsfield Test Equipment LTD., UK) by the strip method according to KS K 0860. An average of five test runs has been reported.

### *5) Structure Analysis*

Structural changes in untreated and lipase-treated PLA nonwovens were evaluated using differential scanning calorimetry (DSC), wide angle X-ray scattering (WAXS), and Fourier transform infrared (FT-IR) analysis. Thermal properties of PLA nonwovens were examined by using DSC (DSC Q 1000, TA instrument, USA) at a heating rate of 20°C$min^{-1}$ over the temperature range 450°C to 300°C. The procedure was repeated twice. The melting temperature ($Tm$) was obtained from the DSC curves of the second heating cycle. The crystallinity of the PLA nonwovens was measured by WAXS diffractometry (Bruker, Germany) under the following operating conditions: 40kV and 45mA at λ1.5406 Å. The relative intensity was recorded in the scattering range (2θ) of 0–40° in steps of 0.02° The infrared spectra of the PLA nonwovens were measured by FT-IR spectroscopy (Spectrum One, Perkin Elmer, USA) on KBr pellets. All samples were analyzed in the range 0–4000$cm^{-1}$ at a resolution of 4$cm^{-1}$.

### *6) Scanning Electron Mircoscopy*

The surfaces of the untreated and lipase-treated PLA nonwovens were analyzed using a scanning electron microscope (SEM, S-4800, HITACHI, Japan) after the samples were plated with platinum.

# III. Results and Discussion

## 1. Lipase Treatment

Enzymes show the highest activity at a certain pH and temperature (Cavaco-Paulo & Guebitz, 2003); therefore, finding the optimal pH and temperature depending on the substrate is essential so that the sub-strates could be changed. <Fig. 2>–<Fig. 4> indicate the hydrolytic activity on PLA fibers as processing conditions were varied over pH values of 7.0–9.0, temper-

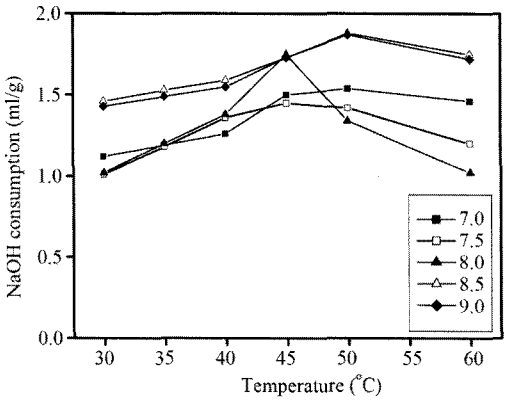



**Fig. 2. NaOH consumption of lipase-treated PLA non-woven against temperature with different pH.**

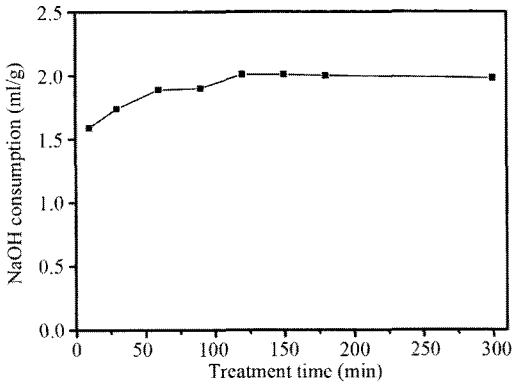



**Fig. 3. NaOH consumption of lipase-treated PLA non-woven depending on treatment time.**

atures of 30–60°C, treatment times of 10–300min, and enzyme concentrations of 1–200%. The hydrolytic activity was used to determine the amount of carboxyl hydrolyzed from ester group and this amount was represented in terms of NaOH consumption. <Fig. 2> shows the effect of pH and temperature on the hydrolytic activity of lipase for PLA fibers in the pH range of 7.0–9.0 and the temperature range of 30–60°C at 50% (o.w.f) lipase concentration for 60min. By lipase treatment, at all pH conditions, the hydrolytic activity increased with temperature; however, after a certain temperature, the hydrolytic activity decreased. In addition, the hydrolytic activity was affected by pH under identical temperature conditions. According Lee and Song (2011), at other pH levels, the hydrolytic activity decreased rapidly because the enzyme-protein structure denatured, and at other temperatures, the enzyme activity either diminished or ceased entirely. As shown in <Fig. 2>, the shapes of the curves for pH 8.0 and those for other pHs show different aspects; this difference can be attributed to the correlation between pH and temperatures. According to Bisswanger, enzyme activity curves depending on pH and temperature resemble a bell-shaped curve; however, they cannot be interpreted in a similar manner. Therefore, aspects of enzyme activity depending pH and temperature can be presented in a different way. In the case of lipase, the enzyme activity is the highest at pH values of 8.5 and 9.0 when treated at 50°C. However, when enzyme treatments, alkaline medium made destroy of the overall structure of the most of enzymes (Bisswanger, 2004), so in this study, both pH 9.0 and pH 8.5, we choose pH 8.5 because it closed neutral pH when lipase treatments. Considering active pH and temperatures, the optimal pH and temperature are 8.5, and 50°C.

<Fig. 3> shows the effect of treatment time on the hydrolytic activity of lipase in PLA fibers in the time range of 10–300min and at a pH value of 8.5 at 50°C with a lipase concentration of 50% (o.w.f). As shown in <Fig. 3>, the hydrolytic activity of lipase increased for 120min, reaching a plateau 120min later. Until the optimum reaction time, the reaction rate of lipase increased linearly, since the reaction occurred proportionally between the enzyme and substrate; after the optimum time, the reaction ceased (Lee & Song, 2011). Therefore, the optimal treatment time is 120min for lipase. In addition, hydrolytic activity that is dependent on treatment time showed little difference for pH and temperature, since the enzyme could be affected by both these two factors and not treatment time (Lee & Song, 2010).

<Fig. 4> indicates the effect of lipase concentration on the hydrolytic activity of PLA fibers in the concentration range of 1–200% (o.w.f) at a pH 8.5 at 50°C for 120min. The hydrolytic activity of lipase showed a linear increase as the lipase concentration increased. Generally, when an enzyme reacts to substrate, the reaction velocity increases linearly if the substrate amount is sufficient (Lee, 2007). In this study, the NaOH consumption increased linearly as enzyme

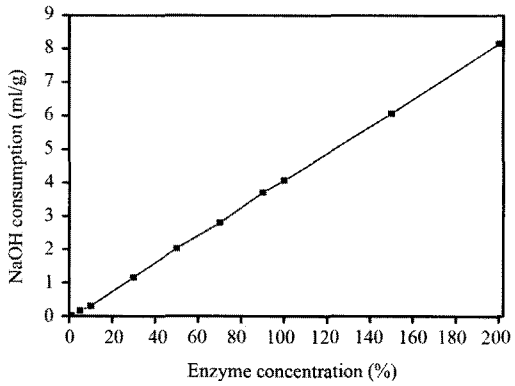



**Fig. 4. NaOH consumption of lipase-treated PLA nonwoven depending on enzyme concentration.**

concentration increased. However, over 50% (o.w.f) of enzyme concentration, enzyme-substrate coagulation was observed, so we concluded that the optimal concentration of lipase was 50% (o.w.f), which did not cause coagulation (Lee & Song, 2011). In all of the experiments described below, the activity for lipase was maximized at 50% (o.w.f) enzyme concentration at 50°C for 120min under pH 8.5; therefore, a change in protein absorbance and a change of interior structure were performed under these conditions.

## 2. Change in Protein Absorbance

<Fig. 5> indicates the change in protein absorbance at 595nm before and after lipase treatment under treatment conditions for this enzyme. In this study, in comparing enzyme protein usage before and after enzyme treatment, we measured the amount of enzyme protein with a Bradford reagent that reacts to proteins. Thus, as the change in protein absorption increases, more of the enzyme is used in the hydrolysis of PLA fibers. As shown in <Fig. 5>, the absorption of protein markedly decreased after lipase treatment, so the usage of lipase in treatment is confirmed in the hydrolysis on PLA fibers.

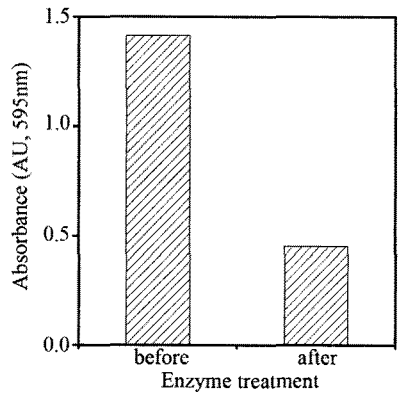



**Fig. 5. Absorbance of treatment solution before and after lipase treatment at 595nm wavelength.**

## 3. Structure Analysis of PLA Nonwovens

Generally, enzyme processing reacts to the surface of fibers, so interior fiber structures do not change. To confirm this, we evaluated the change of the inner structure of a PLA nonwoven. <Fig. 6> and <Table 4> depict the DSC curves for PLA nonwovens, untreated and treated by lipase, under optimum hydrolysis conditions. As shown in <Fig. 6>, an endothermic peak was observed at about 160°C for both untreated and lipase-treated PLA nonwovens. This result concurs with those of previous studies (Cam et al., 1995; Li & Yang, 2006).

<Fig. 7> indicates the WAXS patterns of untreated and lipase-treated PLA nonwovens under optimum

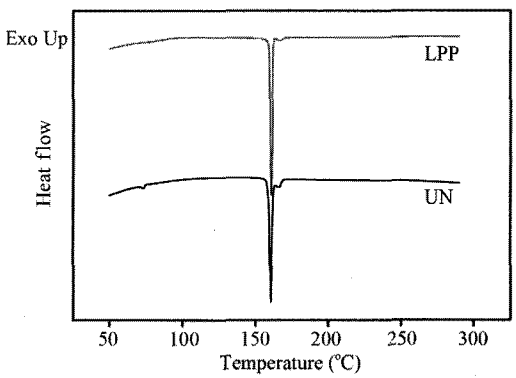



**Fig. 6. DSC curves of untreated and lipase-treated PLA nonwoven.**

**Table 4. Melting point and Heat flow of untreated and lipase-treated PLA nonwoven**

| | Untreated | Lipase treated |
|---|---|---|
| Tm (°C) | 160.7 | 161.1 |
| ΔH (J/g) | 47.9 | 45.0 |

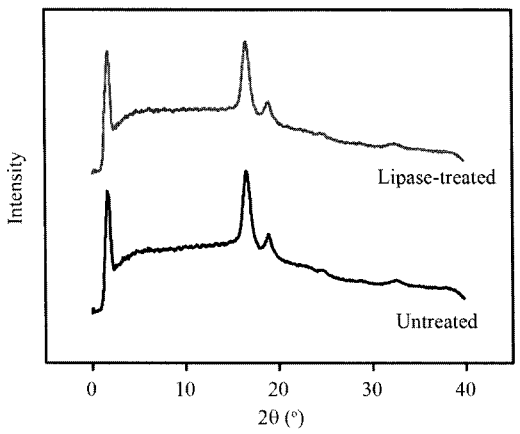



**Fig. 7. XRD of untreated and lipase-treated PLA nonwoven.**

hydrolysis conditions. Two crystalline reflections were observed in the 2θ range of 5–40°. As indicated in <Fig. 7>, the crystal peak of untreated and lipase-treated PLA nonwovens was localized at 2θ=16°, 18°, and it corresponded to the general PLA fiber (Cai et al., 1996). Also, Comparing intensity and width of untreated and lipase-treated PLA nonwovens, by lipase treatment, at 16°, intensity was decreased and width are increased. It means crystalline size was decreased by enzymatic hydrolysis. Therefore, basic crystallinity did not changed by lipase treated, however, crystalline properties little bit increased by hydrolysis of amorphous region (Park & Xanthos, 2009). This is corresponded with previous work. Therefore, there was no change in the crystalline structure due to lipase treatment.

<Fig. 8> shows the FT-IR spectra of untreated and lipase-treated PLA nonwovens under optimum hydrolysis conditions. The FT-IR spectra of untreated PLA nonwovens showed a peak of assigned C=O stretching band at 1,750cm$^{-1}$ indicating the carbonyl group; the FT-IR spectra of PLA fiber exhibited broad peaks at 2800–3000cm$^{-1}$; they were thus assigned to the aliphatic group, a finding which corresponds to the results of previous research (Lin et al., 2007). In addition, the transmittance (%) of lipase-treated PLA nonwovens decreased, since the C=O combination hydrolyzed. Therefore, the results from FT-IR spectra indicated that there was no structural change by lipase treatment, but the intensity decreased a little.

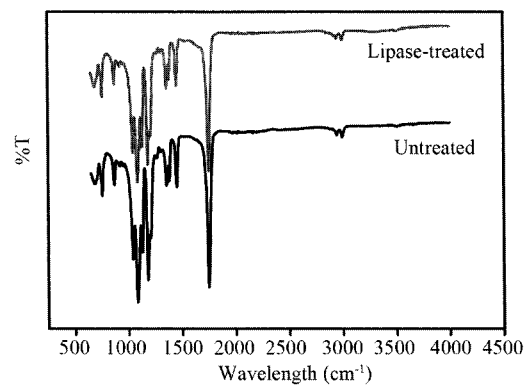



**Fig. 8. FT-IR Spectrum of untreated and lipase-treated PLA nonwoven.**

## 4. Tensile Properties of PLA Nonwovens

<Fig. 9> shows the tensile strength of untreated and lipase-treated PLA nonwovens under optimum hydrolysis conditions. PLA nonwovens used in this study were manufactured with the spun bond method, so the tensile strength in the machine direction (MD) was stronger than that in the cross machine direction (CD). As shown in <Fig. 9>, untreated and lipase-treated PLA nonwovens showed similar tensile strength at a 95% confidence interval. Therefore, there was no fiber damage and physical properties were maintained by enzymatic hydrolysis.

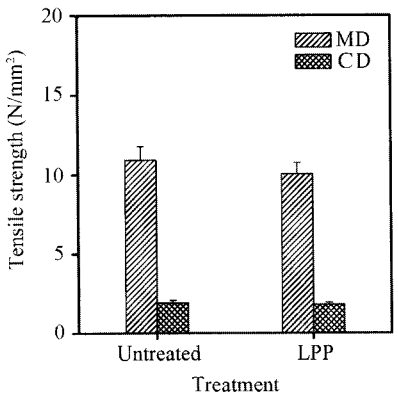
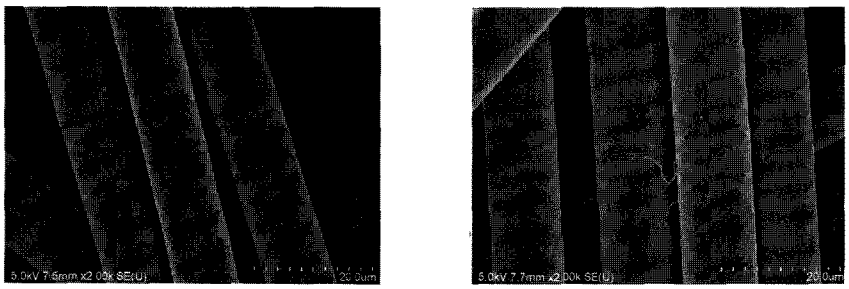



**Fig. 9. Tensile strength of untreated and lipase-treated PLA nonwoven.**
(MD: machine direction, CD: cross machine direction)



(A) (B)

**Fig. 10. SEM micrograph of lipase-treated PLA nonwoven; (A) Untreated, (B) lipase treated.**

### 5. Surface Morphology

<Fig. 10> shows the SEM images of untreated and lipase-treated PLA nonwovens under optimum hydrolysis conditions. Comparing untreated PLA nonwoven (A), lipase-treated PLA nonwoven (B), the former has some fibrils on its surface of its fibers due to enzymatic hydrolysis. Therefore, by lipase treatment, PLA fibers hydrolyzed, but enzymatic treatment did not change the structure or appearance of fabric. Therefore, enzymatic processing with lipase was effective in post-finishing, which can maintain the fiber properties, minimizing fiber damage.

## IV. Conclusions

The purpose of this study was to develop an enzymatic processing method for PLA fibers as an environmentally friendly technology. For this purpose, PLA nonwoven and lipase from *Porcine pancreas* were chosen. The effects of hydrolytic activity depending on the pH, temperature, treatment time, and enzyme concentration on PLA fibers were examined according to the number of carboxyl groups, change in protein absorbance. Furthermore, DSC, WAXS, FT-IR, and SEM were used to evaluate the physical and chemical changes due to the hydrolysis of the PLA nonwovens. The results are as follows:

1. The conditions for the maximum hydrolysis were pH 8.5, 50°C, 120min, and 50% (o.w.f) concentration of lipase. Based on the change of protein absorbance, usage of lipase in enzymatic treatment was confirmed.
2. DSC analysis data for the untreated and lipase-treated PLA nonwovens showed similar trends and had a peak value at 160°C. WAXS and FT-IR studies showed no change in the crystallinity and molecular structure of the PLA nonwovens due to lipase treatment. The surface morphology results for PLA nonwoven showed a few fibrils by lipase treatment.
3. This study investigated enzymatic processing on PLA nonwoven in order to determine the optimum hydrolysis conditions for PLA nonwovens without any change in their mechanical or structural properties. Therefore, enzymatic processing using lipase was confirmed that ecofriendly biotechnology for PLA fibers maintaining its properties.

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