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Biodegradation of Polylactic Acid (PLA) Fibers Using Different Enzymes

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Abstract: The biodegradability of polylactic acid (PLA) nonwovens was evaluated using enzymatic degradation. To evaluate enzyme biodegradation, three enzymes, lipase, esterase, and alcalase, which are known to hydrolyze PLA effectively, were selected. Degradation time was determined under optimal enzyme treatment conditions. Enzymatic degradation affected the width and thickness of PLA nonwovens. In addition, the degree of crystallinity of the PLA nonwovens increased with time for the first 21 days of enzyme biodegradation and then decreased. Alcalase was more efficient than lipase and esterase in degrading the PLA nonwovens. Degradation was found to create cracks on the fiber surface, making the fibers rough. Weight loss and change in tensile strength were not significant, and the observed changes could be due to the cracks on the surface. Our findings propose the mechanism of enzymatic degradation of PLA nonwovens, which might be useful for waste management in the textile industry.

Keywords: polylactic acid (PLA), nonwoven, degradation, enzymatic degradation.

Introduction

The development of biopolymers has been accelerated since environmental regulations for waste management have come into effect. In fact, biodegradable polymers are predicted to be key factors in future industries. Biodegradable polymers can be classified into three groups: (1) natural biopolymers such as cellulose, starch, chitin, and polysaccharides; (2) synthetic polymers, which are produced by polymerization, such as polylactic acid (PLA) and poly(ε-caprolactone) (PCL); and (3) blended polymers such as PCL/starch.[1]

Among these, PLA is one of the best studied. PLA is a linear, aliphatic, thermoplastic polyester of lactic acid (2-hydroxy propionic acid), which is derived from completely renewable sources such as corn.[2-4] Owing to these properties, PLA products are highly marketable and offer advantages such as low unit cost of raw materials; availability of raw materials from renewable, agricultural sources; and high biodegradability. In fact, PLA can be completely hydrolyzed to CO_2 and H_2O by microorganisms in the environment.[5,6]

Because of the advantages they offer, PLA products have several industrial applications.[3,7] In particular, research aimed at enhancing the applicability of the fibers in the textile industry has led to the development of various types of PLA fibers such as nonwoven, woven, and knit fabrics.[2,3,8-11] In addition, they have used extensively in the textile industry as a biomass material because properties of the PLA fiber are similar to those of polyethylene terephthalate (PET).[12] In fact, PLA fibers are said to have the potential to replace conventional petrochemical-based polymers in the textile industry.[12] To apply PLA fibers in clothing materials in the textile industry, understanding the degradation characteristics of PLA fibers and knowing the process of effective degradation method of PLA fibers are important.[13] However, most wastes, such as those from the textile industry, films, plastics, or nonwovens, are not collected and recycled because of the high processing costs.[14]

PLA fibers can degrade in several ways such as photo degradation, oxidative degradation, degradation with soil, or biodegradation. In general, photo degradation does not provide complete degradation because light cannot penetrate the deeper layers of the soil.[15] Oxidative degradation often causes pollution.[1] Degradation with soil can be defined as polymers can degraded by microorganisms within soil.[16] Biodegradation is the decomposition of substances by the action of microorganisms.[17,18] Biodegradation of polymers involves the conversion of polymers into carbon dioxide, water, and biomass by the action of microorganisms or enzymes.[19] Among those methods, for polymer biodegradation, degradation *via* enzyme hydrolysis is the preferred method.[20]

Biodegradation occurs through bio-deterioration, bio-fragmentation, and assimilation.[15,17,21] In general, factors affecting biodegradability are divided into two groups: physical properties of the substance (in this case, polymer) and environmental conditions. Physical properties of polymers include

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molecular structure and conformation, molecular weight, and crystallinity.[13] Environmental conditions include the types of microorganisms, temperature, relative humidity, and pH. Studies on the biodegradation of PLA fibers focus on extent of enzymatic degradation using proteinase K,[22-25] a serine protease from the fungus *Tritirachium album*, depending on factors such as molecular weight, crystallinity,[26,27] pH, or temperature.[28] Enzymatic degradation of PLA films, pellets, and fibers have been limited to specific enzymes or have reported biodegradability on the basis of fiber properties.[24] Reeve *et al*. [25] reported that although enzyme hydrolysis is an effective method, it resulted in loss of surface weight of PLA. In addition, Watanabe *et al.*[29] studied the effect of proteinase K on PLA fibers using gel permeation chromatography (GPC), developed a mathematical model based on the endogenous depolymerization model of degradation, and then compared to the degradability of PLA to that of poly(vinyl chloride) (PVC).

Most of these studies focused single enzymes - lipase, esterase, protease. In addition, most researches about enzymatic degradation using protease which has been studies on enzymatic degradation of PLA is just proteinase K, which is the most expensive protease.[22-25] Moreover; there are no comprehensive reports on the enzymatic degradation of PLA by different enzymes and on the interaction between the different enzymes and the similarities between their effects, especially, on fiber shapes.

Therefore, in this study, we evaluated degradation of PLA nonwovens, which is well used, by lipase from *Candida cylindracea*, esterase from *porcine liver*, and alcalase from *Bacillus licheniformis* - three enzymes that were reported to be effective for the hydrolysis of PLA fibers in a previous study.[10] The time dependence of biodegradation was evaluated under optimal conditions. Bio-deterioration and bio-fragmentation were used as indicators to evaluate biodegradation over short periods. The degree of bio-deterioration was estimated using SEM images, and the degree of bio-fragmentation was estimated from the X-ray diffraction (XRD) patterns. Biodegradability was calculated from weight loss and the change in the length, width, and thickness of the fibers. Our results could be useful for establishing a protocol for biodegradation of PLA nonwovens, which would in turn help in the management of textile industry wastes. Furthermore, these data would also be useful for identification of enzymes that would be effective for degradation of textile industry wastes and discarded clothing materials.

Table I. Characteristics of Nonwove Sample

Sample (%)	Thickness (mm)	Weight (g/m^2)	Manufacturing Method
PLA 100	0.126	30	Spunbond

Experimental

Materials. All PLA nonwovens used in the study were supplied by Toray Industries Inc. (Table I). Lipase (EC 3.1.1.3) from *C. cylindracea*, esterase (EC 3.1.1.1) from pig liver, and alcalase (EC 3.4.21.62) from *Bacillus licheniformis* were used in the study (Table II). These enzymes were used without further purification. Tris (hydroxymethyl) amino methane (pK_a=8.06 at 20 °C, Sigma Chemical Co., USA) was used for buffering. Sodium azide (Junsei Chemicals, Japan) was used as an antifungal agent. Calcium chloride (Junsei Chemicals, Japan) and *L*-cysteine (Yakuri Pure Chemicals Co. Ltd., Japan) were used as activators of enzymatic biodegradation.

Enzymatic Degradation. PLA nonwovens were degraded using lipase, esterase, and alcalase. Strips of PLA nonwovens were prepared (50 [*w*]×150 [*l*]×0.126 [*h*] mm, approximately 0.5 g). As per a previously reported protocol,[10] samples for lipase and esterase biodegradation were placed in separate Erlenmeyer flasks containing 25 mL of TRIS buffer (pH 8.0, 40 °C) with 10% (owing weight of fabric) enzyme concentration, which is the optimum condition for enzymatic hydrolysis, and 10 mM calcium chloride. Samples for alcalase biodegradation were immersed in 25 mL of TRIS buffer (pH 9.5, 60 °C) with 50% (on weight of fabric, owf) enzyme concentration, which is the optimum condition for enzymatic hydrolysis, and 3 mM *L*-cysteine. Sodium azide (0.05 wt%) was added to all reaction mixtures. Biodegradation was evaluated on days 0, 3, 7, 14, and 21. At each indicated day, changes in appearance, weight loss, tensile strength, surface morphology, and crystallinity of control and treated samples were evaluated.

Change in Appearance. Changes in appearance of enzyme-degraded nonwovens were determined based on the length, width, and thickness of the fibers. Changes in length (%) and width (%) of biodegraded and non-biodegraded fabrics were compared. Changes in thickness were measured using a textile thickness gauge (2046F, Mitutoyo, Japan).

Weight Loss. Weight loss of the enzyme-degraded nonwovens was evaluated by determining the dry weight of the

Table II. Properties of Enzymes

Enzyme	Source	Activity	Form	Manufacturer
Lipase (EC 3.1.1.3)	*Candida cylindracea*	2U[a]/mg	Powder	Fluka
Esterase (EC 3.1.1.1)	*Porcine liver*	15U[b]/mg	Crude	Sigma
Alcalase (EC 3.4.21.62)	*Bacillus licheniformis*	2.4AU/g	Liquid	Novozymes

[a]One unit will hydrolyze 1.0 μmol of oleic acid to butyric acid and ethanol per min at pH 8.0 at 25 °C. [b]One unit will hydrolyze 1.0 μmol of ethyl butyrate to butyric acid and ethanol per min at pH 8.0 at 40 °C.

samples. The samples were dried in a conventional drying oven at 105 °C for 90 min, cooled in an automatic desiccator, and then weighed in a closed weighing bottle. The percentage weight loss was calculated as follows:

$$\text{Weight loss (\%)} = \frac{W_1 \times W_2}{W_1} \times 100$$

Where W_1 and W_2 are the dry weights of the samples before and after biodegradation, respectively.

Tensile Strength. The tensile strength of the enzyme-degraded nonwovens was determined using a Universal Testing Machine (H 100KS, Hounsfield Test Equipment LTD., UK) by the strip method according to KS K 0521 and KS K ISO 9073-3. The average of five test runs was reported.

X-Ray Diffraction. The crystallinity of the enzyme-degraded nonwovens was determined using an X-ray diffractometer, (X'pert APD, Philips, USA) under the following operating conditions: 40 kV and 30 mA at λ 1.5406 Å. The relative intensity was recorded in the scattering range (2θ) of 5°-45° in steps of 0.03°. The degree of crystallinity was calculated from the extent ratios of the crystalline area and amorphous area to the integral extent. Mechanical background was removed from the raw data, divided by the crystal peak and amorphous peak, and fit to the Pearson VII function.

Scanning Electron Microscopy. The surface of the enzyme-degraded nonwovens was analyzed using a scanning electron microscope (SEM, S-4800, Hitachi, Japan) after the samples were plated with osmium.

Results and Discussion

Characteristics. Figure 1 shows the changes in length (a) and width (b) of PLA nonwoven samples with the time taken for enzymatic degradation. No significant decreases were observed in the length of the enzyme-degraded PLA nonwovens. Generally, fibers are inflated by degradation. By inflation, the amorphous region of the fiber could be affected. When a fiber was drawn, it orientated by length directions.[30] Therefore, the length of the fiber could have more orientation than width direction. These findings suggested that the length of PLA nonwovens did not change because of inflation. Alcalase-treated PLA nonwoven samples could not be evaluated at 21 days because they were completely degraded. Although there was no significant change in the length of the fibers, some changes were noted in the width of PLA nonwoven samples. The width of the PLA nonwovens decreased considerably 3 days after alcalase digestion and 14 days after esterase degradation. In short, enzyme degradation affects only the width of the PLA fibers. The decrease in width was also evident from the change in the thickness of the PLA nonwovens.

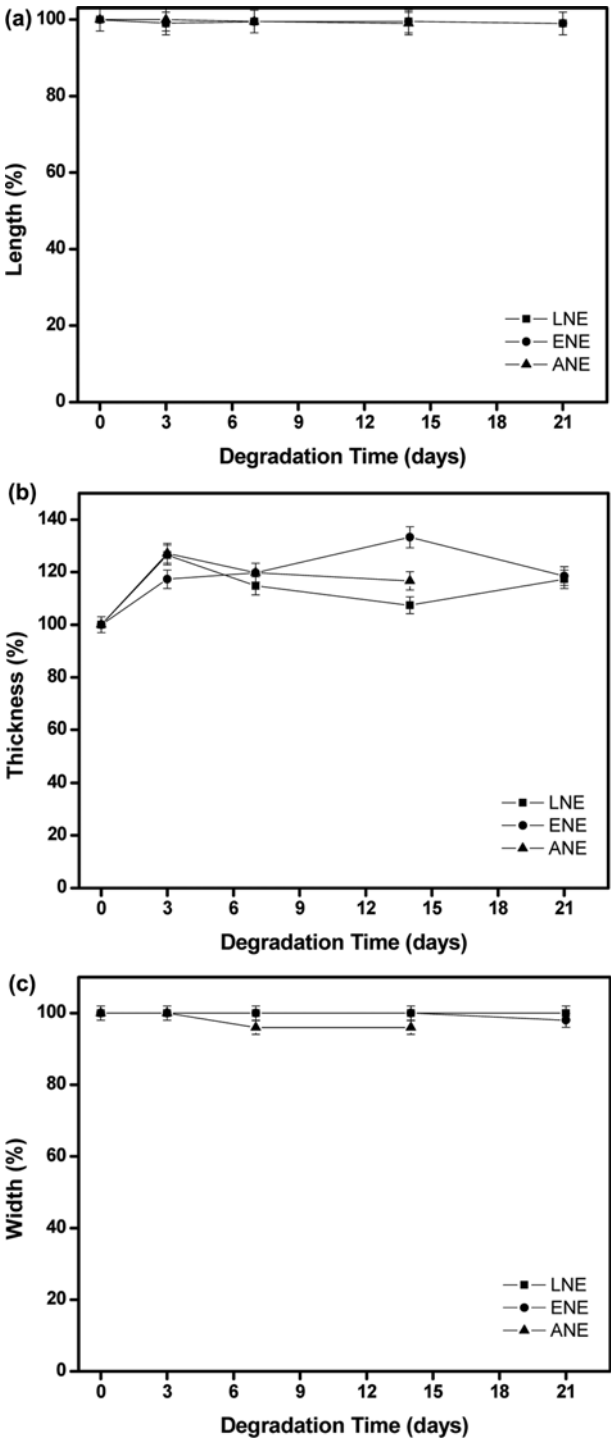

Figure 1. Length (a), thickness (b), and width (c) of PLA nonwovens with respect to duration of enzymatic degradation (LNE: PLA nonwovens degraded by lipase, ENE: PLA nonwovens degraded by esterase, ANE: PLA nonwovens degraded by alcalase).

Figure 1(c) shows the thickness of PLA nonwoven samples against the respective duration of enzymatic degradation. The thickness of the PLA nonwovens digested with lipase increased rapidly for 3 days and then began decreasing up to day 14. However, the thickness of the fibers digested with esterase increased up to day 14 and then began to decrease.

This result matched the change in width. In other words, during inflation, there was no change in the length, while the width decreased. Consequently, shrinkage was expressed as the change in thickness. Further, the thickness of the fibers degraded using alcalase decreased slightly after 3 days, but the fibers were completely degraded after 21 days. Especially, the fiber of alcalase degraded was thickened and shrank by width direction after 3 days through the inflation. The thickness of lipase-samples and esterase-degraded samples was similar after 21 days. This could be due to the process used to manufacture the nonwovens. Because the density of the nonwovens was not uniform, degradation occurred first at weak spot, at dense region. Therefore, there was no significant change in the length, although there were changes in the width and thickness. Based on these findings, it could be reasoned that a fiber thickens when its amorphous regions are degraded, and it thins when the crystalline portions are degraded.

Crystallinity. Figure 2 shows the time dependence of the XRD patterns of PLA nonwoven samples. The crystal peak of untreated and enzyme-degraded samples was localized at 2θ values of 16° and 28°, respectively. A third peak in the lipase- and alcalase-degraded PLA nonwovens was observed at 18.5°, but it was not present in samples degraded with esterase. This result is consistent with the results of Park and Xanthos,[10] who reported a novel third peak at 18.5° indicating morphological changes. This suggests that amorphous PLA crystallized because enzymatic degradation resulted in shorter chains, which are more mobile than longer chains.[17] In addition, according to Gonzalez *et al*.,[31] presence of new peaks could be attributed to oligomeric stereo complex crystal formation, which could explain the change in the crystallinity of PLA nonwovens upon degradation. Among the three enzymes, the hydrolytic activity of lipase was the greatest. Lipases isolated from pig pancreas, *C. cylindracea*, and *R. arrhizus* hydrolyze the amorphous region of the PLA polymer.[32] In case of enzymatic degradation by alcalase, the third peak was quite high because alcalase hydrolysis was conducted at the glass transition temperature of PLA (T_g, 60 °C), which could have affected PLA crystallization.[33] These findings show that lipase and alcalase degradation affects crystallinity more than esterase degradation.

Table III shows the crystallinity of the enzyme-degraded samples. The crystallinity of PLA nonwovens increased from 83% before enzymatic treatment to 88%-92% after enzyme treatment. After 7 days of lipase degradation, crystallinity began decreasing slightly. With esterase and alcalase degradation, crystallinity increased to 88%-92% until day 14, after which it decreased to 82%-85% by day 21. Degradation of the amorphous region of the fibers likely caused crystallinity to increase initially before decreasing as degradation progressed.[15]

Visual Observations. Figure 3 shows surface morphology of enzyme-degraded PLA nonwovens. The results of surface morphologies are related with those of thickness. After 7 days of lipase and esterase degradation, there was no change to the fiber surface. After 14 days, the surface was observed to be rough at some regions, and the weak part of the fiber surface was somewhat squashed because the amorphous region had been degraded. Compared to lipase and esterase degra-

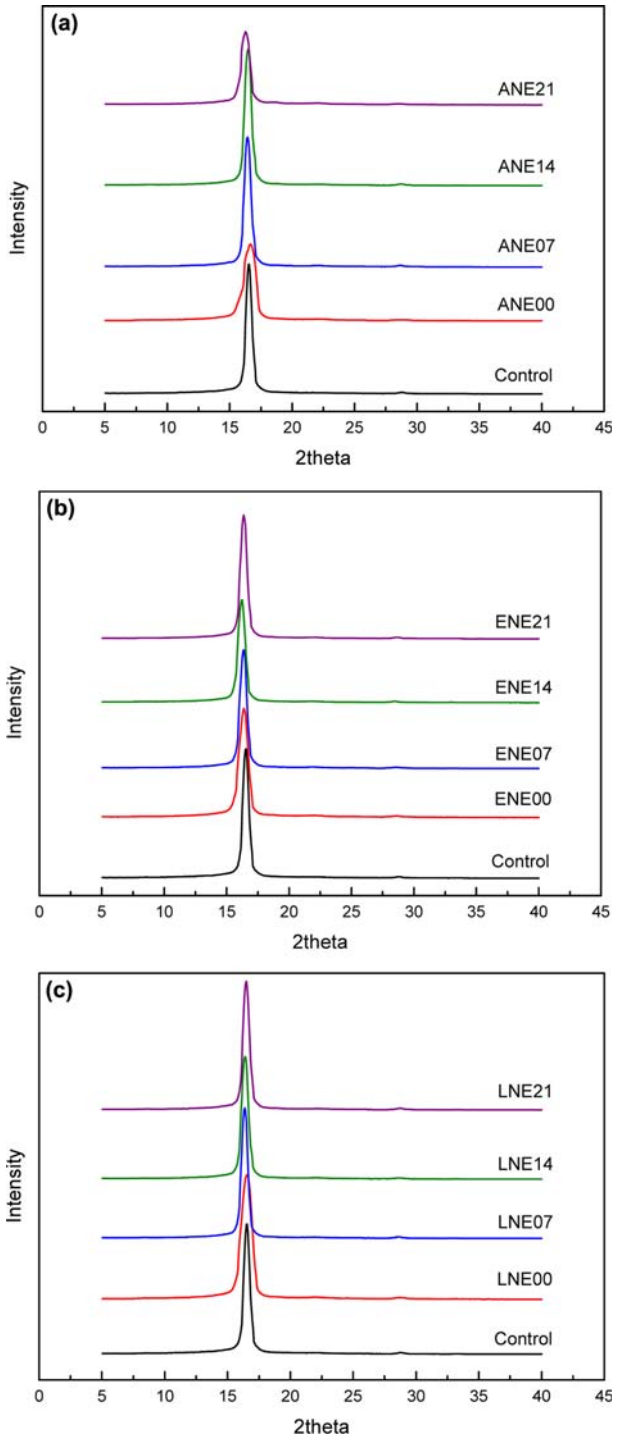

Figure 2. XRD spectra of PLA nonwovens with respect to duration of enzymatic degradation; (a) lipase, (b) esterase, (c) alcalase (LNE: PLA nonwovens degraded by lipase, ENE: PLA nonwovens degraded by esterase, ANE: PLA nonwovens degraded by alcalase).

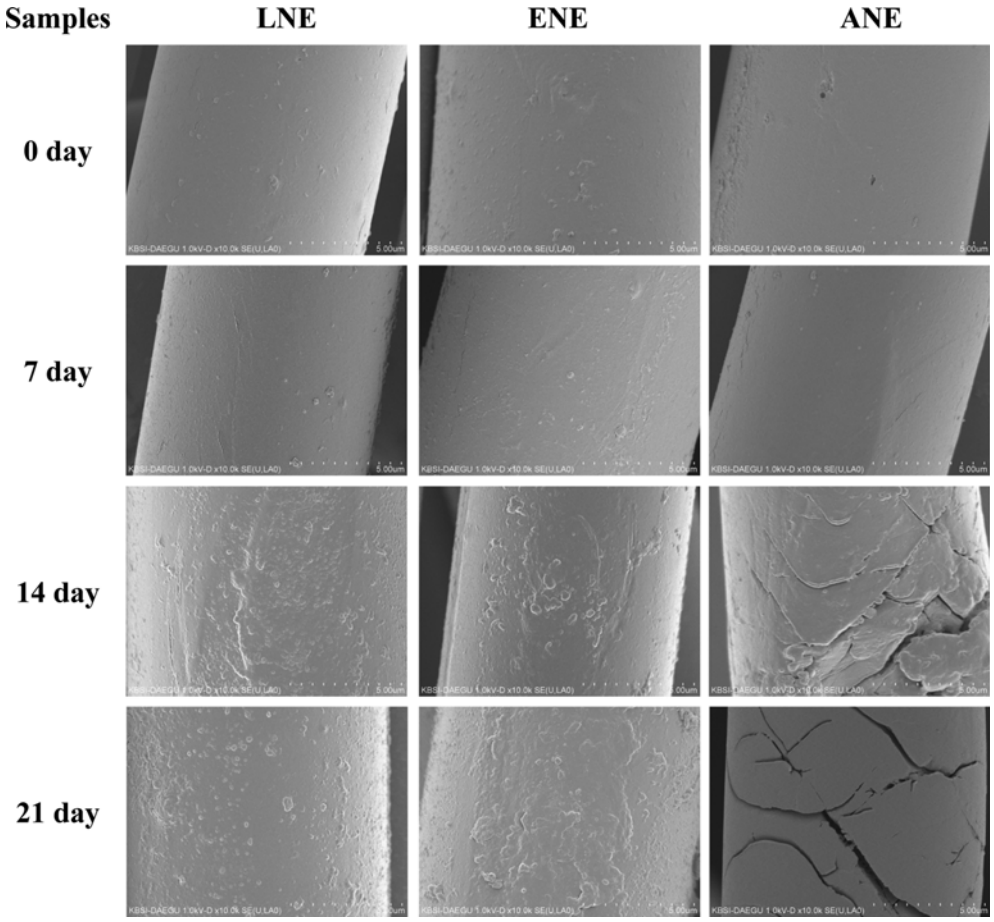

Figure 3. SEM images of lipase-treated PLA nonwovens with respect to duration of enzyme degradation (LNE: PLA nonwovens degraded by lipase, ENE: PLA nonwovens degraded by esterase, ANE: PLA nonwovens degraded by alcalase).

Table III. Degrees of Crystallinity of Samples with Respect to Duration of Enzyme Degradation

Enzyme Degradation		0 day	7 day	14 day	21 day
PLA Nonwoven	Lipase	92	89	89	83
	Esterase	88	89	90	82
	Alcalase	88	89	91	85

dation, alcalase degradation for 14 days resulted in many cracks on the surface of the fibers and in some fibers being cut. After 21 days of alcalase degradation, the cracks intensified, and the fibers thickened. Alcalase degradation of PLA nonwoven samples showed the greatest change in thickness, resulting in deeper cracks, indicating that alcalase degraded the fibers to a greater extent than did lipase and esterase. The SEM images in the figures show the roughened surfaces of the fibers, which could be evidence of bio-deterioration. Bio-deterioration is mainly the result of microorganisms growing on the surface or/and inside of the materials,[34,35] and it is evidence for erosion of PLA fibers.[14] In the bio-deterioration stage, some microbes can adhere and alter the structure and size of pores. As bio-deterioration proceeds, the resistance and durability of the fibers decrease,[17] so that the fibers inflate and thicken. As results, after 21 days of enzymatic bio-deterioration, biodegradation begins.

Weight Loss and Tensile Strength. Figure 4 shows weight loss and the tensile strength during biodegradation of PLA nonwovens with respect to duration of enzyme treatment. The weight loss of the lipase-degraded nonwovens increased about 2.1 fold after 14 days and 3.5 fold after 21 days. There was no weight loss in the esterase-degraded PLA nonwoven samples initially, but 0.80% and 1.40% weight loss was recorded after 7 and 21 days, respectively. The weight loss from alcalase degradation was the lowest at 3 days, but it increased continuously to 25%, about 121 fold, 21 days later. PLA nonwoven samples appeared to be completely biodegraded following alcalase degradation because the fibers lost their molecular conformation. However, after filtration through Whatman paper, weight loss was 25%, not 100%, because residual degraded polymers remained in the solution.

Among the three enzymes tested, alcalase demonstrated the highest biodegrading activity because of its optimal treatment conditions. The optimal treatment conditions were pH

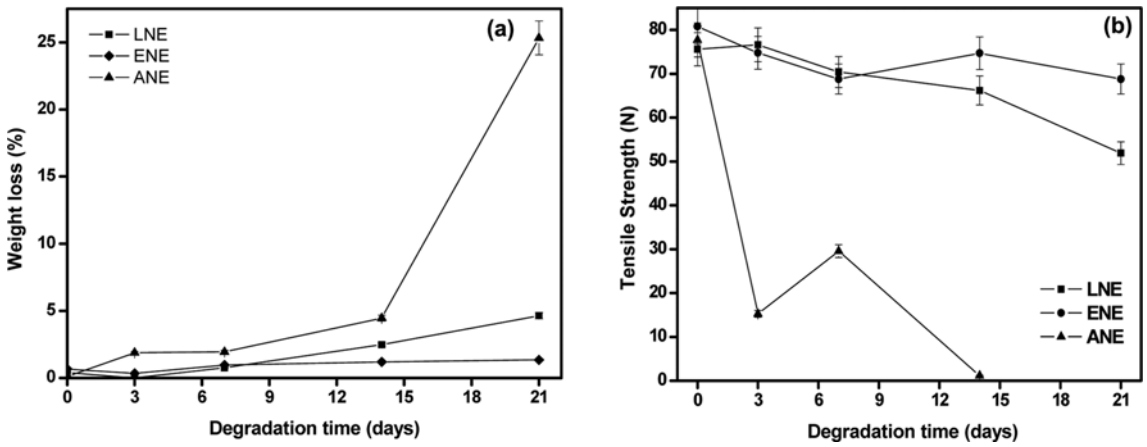

Figure 4. Loss of weight (a) and the tensile strength (b) of PLA nonwovens with respect to duration of enzymatic degradation (LNE: PLA nonwovens degraded by lipase, ENE: PLA nonwovens degraded by esterase, ANE: PLA nonwovens degraded by alcalase).

9.5, 60 ºC for alcalase and pH 8.0, 40 ºC for both lipase and esterase. Typically, PLA fibers are weak in alkaline solution at temperatures above 60 ºC. Alkaline protease isolated from *Bacillus* has appreciable degradation ability.[32,36] In addition, amorphous PLA was degraded by annealing[29] because alcalase degradation occurred at the glass transition temperature around 60 ºC. Further, the weight loss observed coincided with changes in thickness and surface morphology. Alcalase degradation of PLA nonwovens showed the largest change in thickness and resulted in the deepest cracks along with significant weight loss. Degradation of PLA nonwovens proceeded by inflation leading to thickening.

As shown in Figure 4(b), the tensile strength decreased over time. After 21 days, the tensile strength of PLA nonwovens decreased to about 30% following lipase degradation, 15% following esterase degradation, and 100% following alcalase degradation. The tensile strength of alcalase-degraded PLA nonwovens decreased drastically after only 3 days, and after 14 days, the tensile strength could not be measured because the center part of the nonwovens were highly degraded. At 21 days of degradation, alcalase completely degraded the sample. The loss in the tensile strength of esterase-degraded nonwovens was mild compared to that of lipase- and alcalase-degraded nonwovens. Degradation of PLA nonwovens by alcalase was characterized by large weight loss and cracks that occurred without change in crystallinity. Therefore, the loss of tensile strength was likely due to the cracks rather than to changes in crystallinity.

Conclusions

Following enzymatic degradation, the width of the PLA nonwovens changed, with no change in their length. Because of this decrease, the thickness changed by inflation. Of the three enzymes evaluated, alcalase was more efficient than lipase, and biodegradation created cracks on the fiber surface, which led to fiber thickening. A new third peak was observed at 18.5º in the XRD patterns of the lipase- and alcalase-degraded PLA nonwovens. The degree of crystallinity of PLA nonwovens increased with time for 21 days, after which, it decreased to about 3%-9%. Swollen rough structures were observed on the surface of fibers degraded with lipase and esterase for 14 days, indicating bio-deterioration. Cracks occurred after 14 days of alcalase degradation, indicating bio-fragmentation. After 21 days of degradation, the weight loss of PLA nonwovens increased to about 5-fold with lipase and 62-fold with alcalase. The tensile strength of PLA nonwovens decreased about 1.4-fold with lipase, 1.1-fold with esterase, and 100-fold with alcalase after 21 days of degradation. The loss of weight and tensile strength was due to cracks in the surface. Alcalase-degraded PLA nonwovens were degraded more than lipase- or esterase-degraded PLA nonwovens, as demonstrated by the enormous loss of weight and tensile strength. Through this work, the biodegradability of PLA nonwovens by degradation with three enzymes was investigated.

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