Enzymatic Degradation of Poly(ε-caprolactone) Fibers *in vitro*

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ABSTRACT: Poly(ε-caprolactone) (PCL) fibers were enzymatic degraded by a hydrolase *in vitro*. The extent of degradation of PCL fibers was examined by weight loss, mechanical properties loss such as tensile strength and ultimate elongation decrease, and visual observation by scanning electron microscopy. The *in vitro* degradation of PCL fibers was carried out using Lipase F-AP as a hydrolase. A kinetic study on the weight loss of PCL fiber accompanying the enzymatic degradation suggested degradation of PCL fibers gradually from the surface of the fibers into their core. Scanning electron microscopy supported surface, not bulk degradation. The rate of degradation was found to depend on draw ratio and crystallinity of the PCL fibers. Strength loss of PCL fibers in the course of degradation took place faster than weight loss of PCL fibers.
KEY WORDS   Poly(ε-caprolactone) Fiber / Enzymatic Degradation / Lipase F-AP / Mechanical Properties /

Environmental pollution brought about by plastic wastes has become a global problem. One solution to the problem on plastic wastes is application of biodegradable thermoplastics which degrade in soil, sea or lake water, activated sludge, and compost after service life.

It is interesting to consider the use of biodegradable plastics from the viewpoint of environmental applications since environmentally degradable man-made fibers[1] for commodity or industrial uses such as agricultural mulch sheets, fishing nets, and cover stock for disposable diapers are expected to be degraded by enzymes secreted by microorganisms in soil, compost, activated sludge and water.

Poly(ε-caprolactone) (PCL) is an aliphatic polyester that is a relatively stable synthetic polymer under usual condition and is biodegradable under microbial attack, including river and lake waters, sewage sludge, farm soil, paddy soil, creek sediment, pond sediment and compost.[2-4] PCL is a partially crystalline polymer that has a moderately low melting point of 60°C. PCL is susceptible to assimilation by microorganisms such as fungi and bacteria. Environmental degradation of PCL has been extensively studied for biodegradable plastics.[5-9] That the biodegradation of a variety of polyesters have been studied with various enzymes and found that aliphatic polyesters such as PCL were degraded by lipases. It was shown that the amorphous regions of PCL films are degraded more readily than crystalline areas by scanning electron microscopy visualization[10-12] as well as thermal analysis.[12]

In a previous paper, environmental degradation of PCL fibers with various draw ratios in soil, activated sludge and seawater was reported.[13] The extent of degradation was examined by weight loss, loss of mechanical properties, and visual observation by scanning electron microscopy. The rate of degradation was found to

depend on the draw ratio and crystallinity of the PCL fibers with surface erosion of amorphous regions more readily than crystalline regions. The amorphous part of the film-blown PCL samples is degraded prior to the crystalline part in a biotic environment.[11,12]

Enzymatic degradation of the PCL fibers with various draw ratios *in vitro* by Lipase F-AP was studied to compare the behavior of the environmental degradation,[13] and the effects of the crystallinity of the fiber structure on the rate of biodegradation are discussed.

EXPERIMENTAL

Materials

PCL monofilaments were prepared by melt-spinning at 210°C. PCL monofilament of TONE P-787 (The trade name of the PCL polymer by Union Carbide Co., Mn of about 80000) were manufactured by Unitika Ltd., and kindly supplied for the present study. Tough and high tenacity PCL monofilaments with almost same diameters ($280 \pm 5\ \mu$m) and different draw ratios (undrawn, 5.0 and 9.0) were prepared. The structure and properties of the PCL monofilaments were reported in detail previously.[14]

Degradation of PCL Fibers in vitro

Enzymatically degradation studies *in vitro* were carried out using Lipase F-AP (Amano Pharmaceutical Ltd.) from *Rhizopus* sp. as hydrolase. Lipases (triacylglycerol acylhydrolase, EC 3.1.1.3) are widely found in animals, plants, and microorganisms, Lipases from microorganisms are diversified in enzymatic properties and substrate specificities.[14] Lipase F-AP was found to have high specificity toward PCL hydrolysis.[15] The enzyme was dissolved in phosphate buffer solution (PBS) to yield solutions of known enzyme concentrations. PCL fibers were placed in excess enzyme solution kept at pH 7.0 and 25°C, and removed from solution at appropriate

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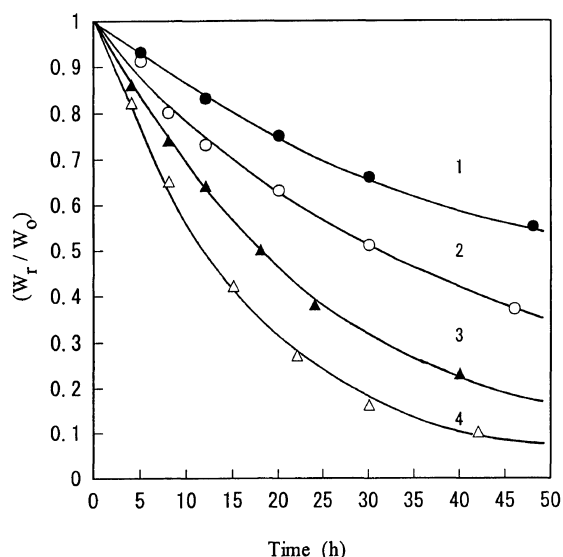†To whom correspondence should be addressed.

Figure 1. Dry weight ratio W_r/W_o of undrawn PCL fibers as a function of Lipase F-AP degradation time (h) at 25℃ and pH 7.0: (1) (●) $[E] = 0.10$ wt%, (2) (○) $[E] = 0.15$ wt%, (3) (▲) $[E] = 0.23$ wt%, and (4) (△) $[E] = 0.30$ wt%.
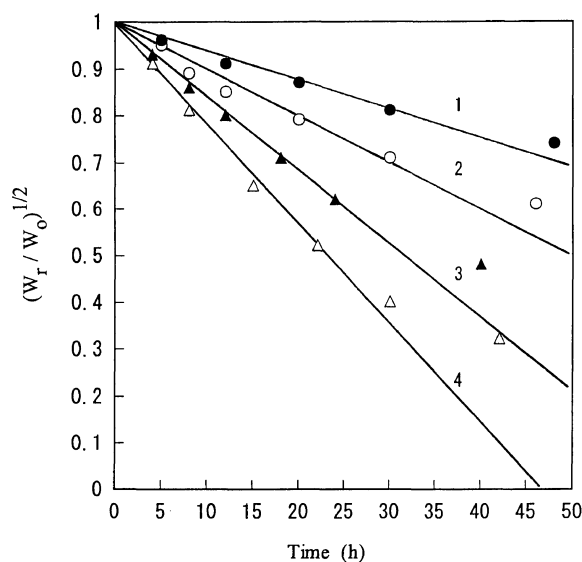

Figure 2. $(W_r/W_o)^{1/2}$ of undrawn PCL fibers as a function of Lipase F-AP degradation time (h) at 25℃ and pH 7.0: (1) (●) $[E] = 0.10$ wt%, (2) (○) $[E] = 0.15$ wt%, (3) (▲) $[E] = 0.23$ wt%, and (4) (△) $[E] = 0.30$ wt%.

times. After vacuum drying at 40℃ to constant weight, the remaining PCL fibers were weighed. The tensile strength of these fibers was measured for five samples of each fiber. Straight-pull tensile strength was determined with a Tensilon UTM-II-20 (Toyo-Boldwin Co.) at an elongation rate of 40% per min.

Before and after degradation, the surfaces of the PCL fibers were observed by field emission scanning electron microscopy (SEM), S-4000 (Hitachi Ltd.) with 4-kV acceleration after Au/PD coating with an ion coater.

RESULTS AND DISCUSSION

Degradation of Undrawn PCL Fibers in vitro

For the first time, weight decrease of the undrawn PCL fibers subjected to degradation with Lipase F-AP was observed at pH 7.0 and 25℃ in PBS. Figure 1 illustrates the dry weight ratio W_r/W_o of the PCL fiber samples as a function of degradation time in various enzyme concentrations. The rate of degradation depended on the enzyme concentration.

It is likely that the enzymatic degradation of the PCL fiber takes place from the surface, since enzyme molecule are difficult to diffuse into the fiber interior because of the large size of enzyme molecule and rather low water content of swollen fiber. The molecular weight of Lipase F-AP is around 30000 and water content of the PCL fiber is less than 10%. If enzymatic degradation takes place from the fiber surface, the rate of weight decrease should be proportional to total surface area of the fibers. This leads to the following equation with the rate constant k as derived in the previous paper:[16,17]

$$\left(\frac{W_r}{W_o}\right)^{\frac{1}{2}} = 1 - \frac{kt}{r_o d_o} \qquad (1)$$

where W_o and W_r are weights of the fiber before and after degradation for time t, respectively, and r_o $(=0.280$ mm) and $d_o (=1.14$ $)^9$ are the radius and the density of the starting fibers, respectively. Figure 2 shows plots of
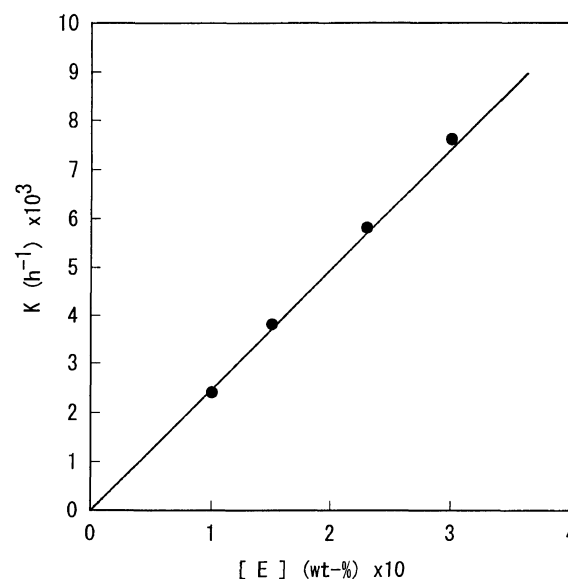

Figure 3. Rate constant, k, for enzymatic degradation of undrawn PCL fibers as function of the concentration of Lipase F-AP at 25℃ and pH 7.0 in PBS.

$(W_r/W_o)^{1/2}$ against t recalculated from the data given in Figure 1. A linear relationship was obtained, at least, at the initial stage of degradation. This linearity of the plot supports the assumption that degradation of the undrawn PCL fibers takes place from the surface into the core. This degradation mode is quite different from that of the poly(glycolic acid) fiber which undergoes degradation almost homogeneously throughout a cross-section of a fiber from the beginning of degradation. Rate constants of degradation for PCL fibers obtained from the initial slopes of the plots in Figure 2 are shown in Figure 3. The k for the undrawn PCL fibers depends on enzyme concentration. As seen in Figure 3, the data almost fit a straight line. Although the degradation proceeds heterogeneously, it obeys first-order kinetics similar to usual
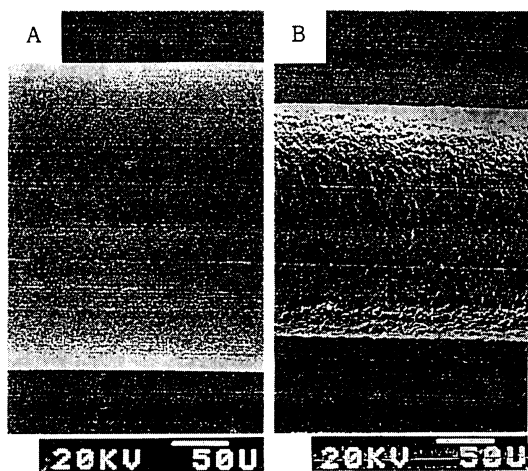
Figure 4. Scanning electron micrographs of undrawn PCL fibers degraded by Lipase F-AP ($[E]=0.15$ wt%) in 0.1 M PBS at 25℃ and pH 7.0: (A) original, (B) 48 h degradation.
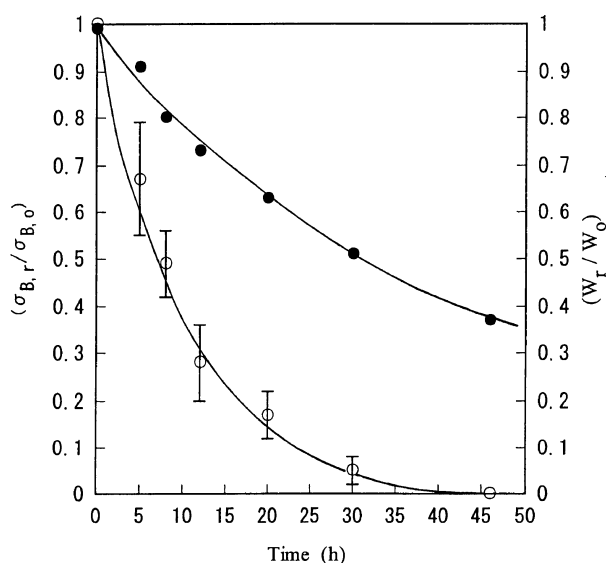

Figure 6. Decrease of relative tensile strength $\sigma_{B,r}/\sigma_{B,o}$ (○) and weight W_r/W_o (●) for drawn PCL fibers (DR=5.0) degraded in 0.1 M PBS at 25℃ and pH 7.0 as a function of Lipase F-AP degraded time (h) ($[E]=0.30$ wt%, $\sigma_{B,o}=2200$ g).

Figure 5. Decrease of relative tensile strength $\sigma_{B,r}/\sigma_{B,o}$ (○) and weight W_r/W_o (●) for undrawn PCL fibers degraded in 0.1 M PBS at 25℃ and pH 7.0 as a function of Lipase F-AP degradation time (h) ($[E]=0.15$ wt%, $\sigma_{B,o}=595$ g).

Figure 7. Decrease of relative tensile strength $\sigma_{B,r}/\sigma_{B,o}$ (○) and weight W_r/W_o (●) for drawn PCL fibers (DR=9.0) degraded in 0.1 M PBS at 25℃ and pH 7.0 as a function of Lipase F-AP degraded time (h) ($[E]=0.30$ wt%, $\sigma_{B,o}=2850$ g).

homogeneous enzymatic reactions.

Surface Observations

As an example of scanning electron micrographs (SEM) of the surface of degraded fibers, the surface of undrawn PCL fibers before and after degraded is shown in Figure 4. Apparently, fiber diameter decreases with degradation, indicating enzymatic action *in vitro* to proceed from the surface of PCL fibers.

Tensile Strength of Degraded PCL Fibers

In Figure 5, decrease in tensile strength of PCL fiber with enzymatic degradation is compared with weight loss for undrawn PCL fibers. Both parameters are expressed relative to those before enzymatic degradation. As seen in Figure 5, the curve of decrease of tensile strength does not fit the weight loss curve. If enzymatic degradation of the PCL fibers results solely in decrease
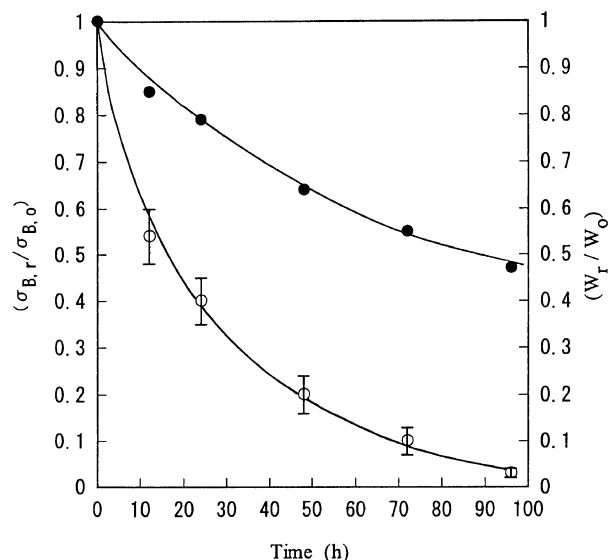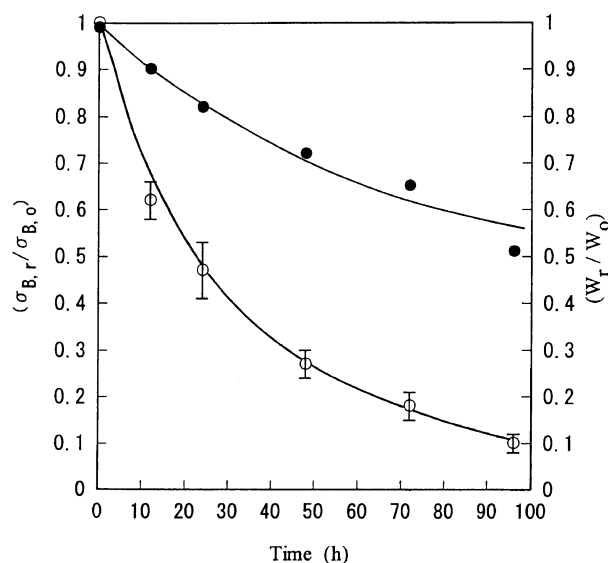
in fiber diameter, the two curves theoretically should fit each other. Numerous possible factors account for the poor fitting, such as defects generated during enzymatic degradation. A small defect has large effect on the strength of fibers but small effect on fiber weight. Undrawn PCL fibers lost mechanical properties faster than their wight loss, as seen in Figure 5. This might be caused by tensile strength being as more sensitive measure of the extent of degradation than weight loss, because failure occurs at cracks or stress concentration points.

To understand the crystallinity of PCL fibers in enzymatic degradation, similar tests were carried out for PCL fiber with different draw ratios, but with the same radius. Experimental data of decrease of relative tensile

strength and weight loss for drawn PCL fibers are summarized in Figures 6 (DR=5.0) and 7 (DR=9.0), respectively. The crystallinity index of PCL fibers is 40.0% for undrawn fiber, 63.3% for drawn fiber of DR=5.0, and 69.3% for drawn fiber of DR=9.0.[14] The initial rate of enzymatic degradation of undrawn PCL fibers was more than six times that of a 5.0 times drawn one with the same enzyme concentration probably due to lower crystallinity. This suggests that enzymes attack preferentially an amorphous or less-ordered region over a crystalline or more-ordered regions because the enzymes migrate more readily into the less-ordered regions than more-ordered regions.

CONCLUSION

It was suggested from a kinetic study on weight loss of the PCL fibers with enzymatic degradation *in vitro* by Lipase F-AP that degradation proceeds gradually from the surface of fibers into the core. The observed rate of weight loss was in good agreement with that predicted from enzymatic surface erosion. This was also supported by scanning electron microscopy. Though the rate of decrease in tensile strength of fibers through enzymatic degradation *in vitro* was higher than that of weight loss, this feature was quite different from that for non-enzymatically degradable fibers such as poly(glycolic acid) fiber, with rather homogeneous degradation throughout a cross-section of fibers from the beginning of degradation. Enzymatic degradability was depressed on increasing crystallinity by drawing. Surface erosion with weight loss by enzymatic degradation appears to be the primary mechanism similar to environmental degradation of PCL fibers.

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