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STUDIES ON BIODEGRADABLE POLY(HEXANO-6-LACTONE) FIBERS. PART 3. ENZYMATIC DEGRADATION IN VITRO

(IUPAC Technical Report)

Prepared for publication by

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Studies on biodegradable poly(hexano-6-lactone) fibers.

Part 3. Enzymatic degradation in vitro

(IUPAC Technical Report)

Abstract: Poly(hexano-6-lactone) (PCL*) fibers were enzymatically 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 decreases, and visual observations by scanning electron microscopy. The in vitro degradation of PCL fibers was carried out using a lipoprotein lipase (Lipase-PS) as a hydrolase. The kinetic study on the weight loss of PCL fiber accompanying the enzymatic degradation suggested that the degradation of PCL fibers gradually takes place from the surface, not bulk degradation. The rate of degradation was found to depend on draw ratio and crystallinity of the PCL fibers. The strength loss of PCL fibers in the course of degradation took place faster than the weight loss of PCL fibers. Sonic velocity measurements as well as dynamic mechanical properties of PCL fibers were also examined as a function of weight loss of sample fibers with Lipase-PS treatments. It was shown that sonic velocity and value of loss tangent δ changed steeply for undrawn PCL fiber in the first step with enzymatic digestion.

INTRODUCTION

Environmental pollutions brought about by plastic wastes have become a global problem. One of the solutions to the problem of plastic wastes is an application of biodegradable thermoplastics, which degrade in soil, sea or lake water, activated sludge, and compost after their service life has been over. It is interesting to consider the use of biodegradable plastics from the viewpoint of environmental applications that 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(hexano-6-lactone) (PCL*) is an aliphatic polyester that is a relatively stable synthetic polymer under usual conditions and is biodegradable under microbial attack, including river and lake waters, sewage sludge, farm soil, paddy soil, creek sediment, roadside 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 being assimilated by microorganisms such as fungi and bacteria. Environmental degradation of PCL has been extensively studied for the biodegradable plastics [5–9]. The biodegradation of a variety of polyesters was also studied with various enzymes, and it was found that aliphatic polyesters such as PCL were degraded by lipases. Furthermore, it was shown that the amorphous regions of PCL films are degraded more readily than crystalline regions by scanning electron microscopy (SEM) visualization as well as thermal analysis [10–12].

In the previous paper, the environmental degradation of PCL fibers with various draw ratios in soil, activated sludge, and sea water is reported [13]. The extent of degradation was examined by weight loss, loss of mechanical properties, and visual observations by SEM. The rate of degradation was found

*The name 'ε-caprolactone' is commonly used rather than the IUPAC nomenclature 'hexano-6-lactone'; hence the abbreviation PCL, based on the former, is used throughout this Technical Report.

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

In the present study, enzymatic degradation of the PCL fibers with various draw ratios in vitro by Lipase-PS to compare the behaviors of the environmental degradation [13], and the effects of the crystallinity and the fiber structure on the rate of biodegradation are discussed.

EXPERIMENTAL

Materials

The PCL monofilaments were prepared by melt-spinning at 210 °C. The PCL monofilaments of TONE P-787 (the trade name of the PCL polymer by Union Carbide, M_n of about 80 000) were manufactured by Unitika. Tough and high-tenacity PCL monofilaments with almost same diameters ($280 \pm 5 \mu\text{m}$) and different draw ratios (undrawn to 9 times drawn) were prepared. The structure and properties of the PCL monofilaments were listed in Table 1 [14].

Table 1 Physical properties of PCL fibers [14].

Draw ratio	Density g/cm^3	$t_m/^\circ\text{C}$	Degree of orientation	Crystallinity index (%)
DR = 1 (undrawn)	1.1403	59	0.3368	40.0
DR = 5 (drawn)	1.1438	60	0.9684	63.3
DR = 7 (drawn)	1.1446	62	0.9767	65.0
DR = 9 (drawn)	1.1484	64	0.9886	69.3

Degradation of PCL fibers in vitro

Enzymatical degradation studies in vitro were carried out using Lipase-PS (Amano Pharmaceutical) from *Pseudomonas fluorescense* as a hydrolase. Lipases (triacylglycerol acylhydrolase, EC 3.1.1.3) are widely found in animals, plants, and microorganisms. In particular, microorganisms are diversified in their enzymatic properties and substrate specificities [14]. Among them, Lipase-PS was found to have high specificities toward PCL hydrolysis. The enzyme was dissolved in phosphate buffer solution (PBS) to yield solutions of known enzyme concentrations. The PCL fibers were placed in excess of the enzyme solution kept at pH 7.0 and 25 °C, and then removed from the solution at appropriate time intervals. After vacuum drying at 40 °C to constant weight, the remaining PCL fibers were weighed.

Tensile tests

The tensile strength of these fibers was measured for five samples of each fiber. The straight-pull tensile strength was determined with a Tensilon UTM- II-20 (Toyo-Boldwin) at an elongation rate of 40 % per minute.

Surface observations

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

Measurements of densities

Densities of PCL fibers before and after Lipase-PS treatments were measured by a density gradient column of NaCl aqueous solution at 25 °C. After 4 h floatation, densities were calculated from the calibration curve by standard floats.

Measurement of sonic velocity

The velocity of longitudinal wave in PCL fibers was determined by measuring the transit time of a sonic pulse at a frequency of 10 kHz. The measurements were conducted at 23 °C. The length of specimen between the transmitting and receiving transducers were varied from 150 to 250 mm.

Measurement of dynamic viscoelasticity

Dynamic viscoelastic properties of PCL fibers before and after Lipase-PS treatments were measured as a function of temperature by a nonresonant forced-vibration type apparatus, Rheovibron DDV-25FP (Orientec) at a heating rate of 1 °C/min.

RESULTS AND DISCUSSION

Degradation of PCL fibers in vitro

For the first time, the weight decrease of undrawn (DR = 1) and drawn (DR = 5) PCL fibers subjected to degradation with Lipase-PS was observed at pH 7.0 and 25 °C in PBS. Figures 1 and 2 illustrate the dry weight ratio W_t/W_0 , in which W_0 and W_t are the weight of the fiber before and after degradation for time t , of undrawn and drawn PCL fiber samples as a function of the degradation time in various enzyme concentrations [E] from 0.05 to 0.20 %. It is shown that the rate of degradation depends on the enzyme concentration, respectively.

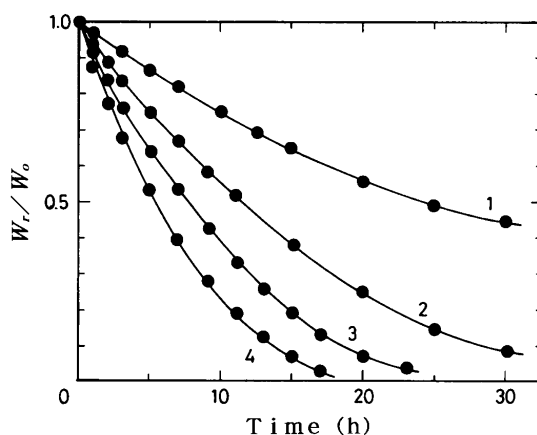


Fig. 1 Dry weight ratio W_t/W_0 of undrawn PCL fiber as a function of Lipase-PS degradation time (h) at 25 °C and pH 7.0: (1) [E] = 0.05 %, (2) [E] = 0.10 %, (3) [E] = 0.15 %, and (4) [E] = 0.20 %.

It is likely that the enzymatic degradation of the PCL fiber takes place from its surface in spite of the presence of water inside the fiber, since the enzyme molecule will be difficult to diffuse into the fiber interior owing to the large size of the enzyme molecule and the rather low water content of the swollen fiber. The molecular weight of Lipase-PS is around 32 000, and the water content of the PCL fiber is

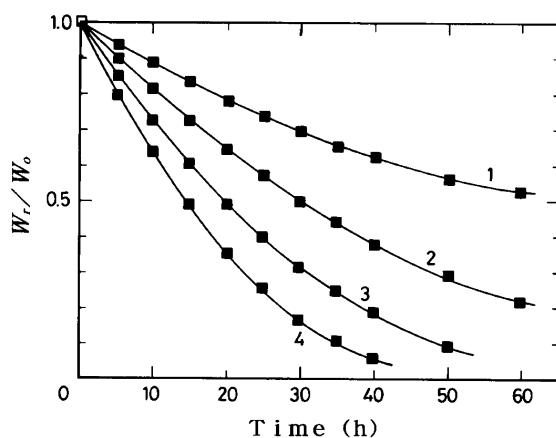


Fig. 2 Dry weight ratio W_r/W_0 of drawn PCL fiber (DR = 5) as a function of Lipase-PS degradation time (h) at 25 °C and pH 7.0: (1) [E] = 0.05 %, (2) [E] = 0.10 %, (3) [E] = 0.15 %, and (4) [E] = 0.20 %.

less than 10 %. If the enzymatic degradation takes place from the fiber surface, the rate of weight decrease should be proportional to the total surface area of the fibers. This assumption leads to the following equation with the rate constant k as derived in the previous paper [16,17]

$$(W_r/W_0)^{1/2} = 1 - kt/r_0 d_0 \quad (1)$$

where r_0 and d_0 ($= 1.14$) are the radius and the relative density of the starting fiber, respectively. Figures 3 and 4 show the plots of $(W_r/W_0)^{1/2}$ against t recalculated from the data given in Figs. 1 and 2, respectively. A linear relationship is obtained, at least, in the initial stage of the degradation. This linearity of the plot supports the assumption that the degradation of PCL fibers takes place from the fiber surface into the core. This degradation mode is quite different from that of the poly(glycolic acid) fiber, which undergoes degradation almost homogeneously throughout the cross-section of the fiber from the beginning of degradation. The rate constants of the degradation for the PCL fibers obtained from the initial slopes of the plots in Figs. 3 and 4, respectively, are shown as a function of the enzyme concentration [E] in Fig. 5. It is shown that the k value for PCL fibers depends on the enzyme concentration. As can

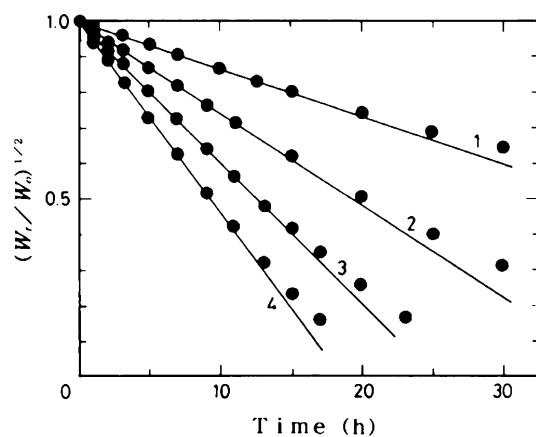


Fig. 3 $(W_r/W_0)^{1/2}$ of undrawn PCL fiber as a function of Lipase-PS degradation time (h) at 25 °C and pH 7.0: (1) [E] = 0.05 %, (2) [E] = 0.10 %, (3) [E] = 0.15 %, and (4) [E] = 0.20 %.

be seen in Fig. 5, the data almost fit a straight line. Although this degradation proceeds heterogeneously, it obeys first-order kinetics similar to the usual homogeneous enzymatic reaction.

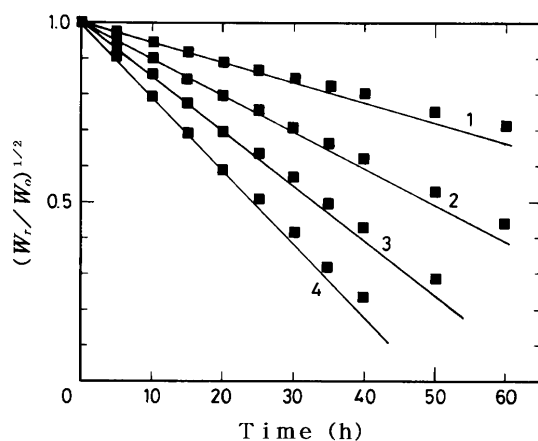


Fig. 4 $(W_t/W_0)^{1/2}$ of drawn PCL fiber (DR = 5) as a function of Lipase-PS degradation time (h) at 25 °C and pH 7.0: (1) [E] = 0.05 %, (2) [E] = 0.10 %, (3) [E] = 0.15 %, and (4) [E] = 0.20 %.

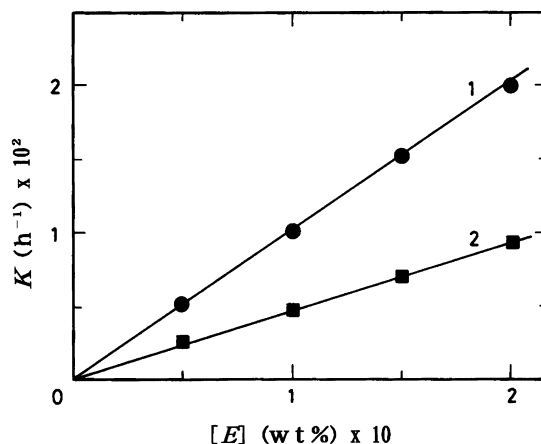


Fig. 5 The rate constant, k , for enzymatic degradation of PCL fibers as a function of concentration of Lipase-PS at 25 °C and pH 7.0 in PBS: (1) (●) undrawn (DR = 1), (2) (■) drawn (DR = 5) fibers.

Surface observations

As an example of scanning electron micrographs of the surface of the degraded fibers, the surface of undrawn (DR = 1) PCL fiber before and after degraded is shown, respectively, in Fig. 6. Apparently, the fiber diameter decreases upon degradation, indicating that the enzymatic reaction *in vitro* proceeds from the surface of the PCL fibers.

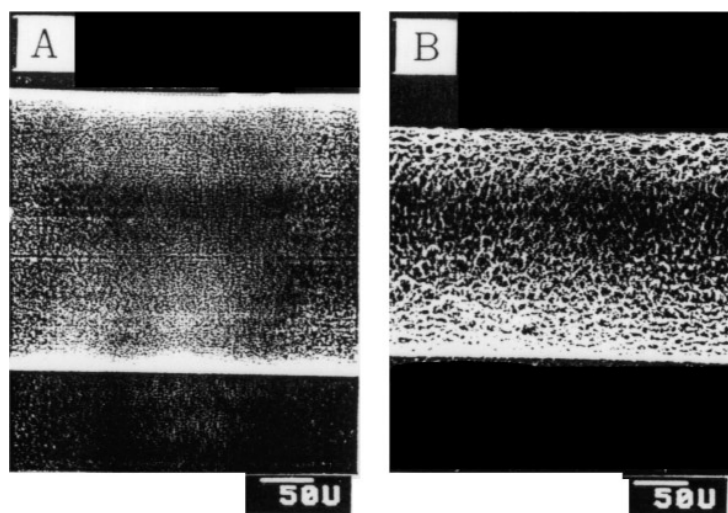


Fig. 6 Scanning electron micrographs of undrawn PCL fiber degraded by Lipase-PS ($[E] = 0.10\%$) in 0.1 M PBS at 25 °C and pH 7.0: (A) original, and (B) 10 h degradation.

Tensile strength of degraded PCL fibers

In Fig. 7, the decrease in the tensile strength of PCL fiber upon enzymatic degradation is compared with the weight loss for the undrawn PCL fibers. Both of them are expressed as values relative to those before enzymatic degradation. As seen in Fig. 7, the curve of decrease of tensile strength does not fit the weight loss curve. If the enzymatic degradation of the PCL fibers results solely in the decrease in the fiber diameter, these two curves should fit theoretically each other. Numerous possible factors could account for the poor fitting. One of them is the defect generated during enzymatic degradation. A small defect has a large effect on the strength of fibers but should not influence the fiber weight. The undrawn PCL fibers lost their mechanical properties faster than their weight loss, as can be seen in Fig. 7. This might be caused by the fact that the tensile strength is a more sensitive measure of the extent of degradation than weight loss, because failure occurs at cracks or stress concentration points in the samples.

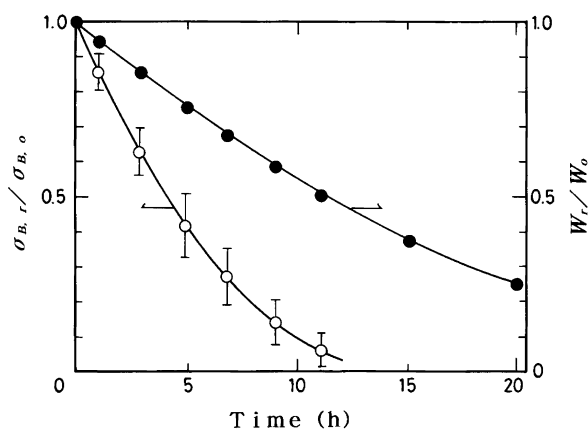


Fig. 7 Decrease of relative tensile strength $\sigma_{B,t}/\sigma_{B,o}$ (\circ) and weight W_t/W_0 (\bullet) for undrawn PCL fiber degraded in 0.1 M PBS at 25 °C and pH 7.0 as a function of Lipase-PS degradation time (h). ($[E] = 0.10\%$, $\sigma_{B,o} = 595$ g.)

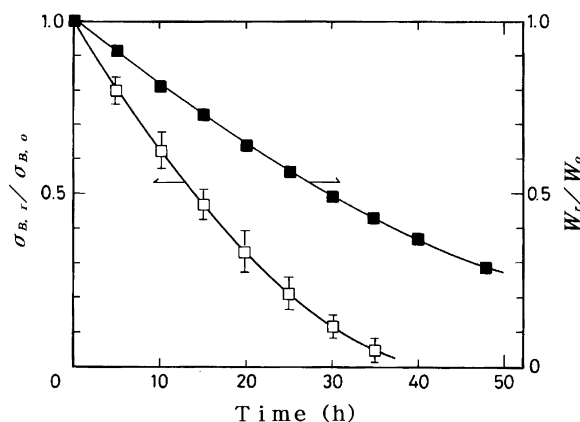


Fig. 8 Decrease of relative tensile strength $\sigma_{B,t}/\sigma_{B,o}$ (\square) and weight loss W_t/W_o (\blacksquare) for drawn PCL fiber (DR = 5) degraded in 0.1 M PBS at 25 °C and pH 7.0 as a function of Lipase-PS degradation time (h). ($[E] = 0.10\%$, $\sigma_{B,o} = 2200$ g.)

In order to understand the effects of the crystallinity and the orientation of the PCL fiber on enzymatic degradation, the similar tests were carried out for drawn (DR = 5) PCL fiber. Experimental data of decrease of the relative tensile strength and weight loss for drawn (DR = 5) PCL fibers are shown in Fig. 8. As shown in Table 1, the crystallinity index of PCL fibers, evaluated by wide-angle X-ray diffraction, were 40.0 % for undrawn (DR = 1) and 63.3 % for drawn (DR = 5) fibers. The initial rate of enzymatic degradation of undrawn PCL fiber is estimated to be more than 5 times faster than that of drawn (DR = 5) fiber with the same enzyme concentration probably due to lower crystallinity. This suggests that the enzymes attack preferentially the amorphous or less-ordered regions than the crystalline or more-ordered regions because the enzymes are able to migrate more readily into the less-ordered regions than the more-ordered regions.

Change of crystallinity with degradation

Measurement of density ρ of Lipase-PS treated PCL fibers were conducted. The weight fraction crystallinity χ_w was evaluated using the equation

$$1/\rho = \chi_w/\rho_c + (1 - \chi_w)/\rho_a \quad (2)$$

where $\rho_c = 1.200$ g/cm³ is the crystalline density [18] of PCL and $\rho_a = 1.021$ g/cm³ is the amorphous density of PCL.

Figure 9 shows the change of crystallinity χ_w of PCL fibers with the weight loss after degradation. In the case of undrawn PCL fiber, the rate of increase of crystallinity is at first quite fast, but with increasing weight loss it becomes constant. Undrawn PCL fiber shows the crystallinity χ_w of 0.54 and the χ_w of degraded fiber is about 0.58 at $W_t/W_o = 0.987$. This suggests that the recrystallization occurred at the first stage of enzymatic degradation of amorphous chains in undrawn fiber. In the case of drawn PCL fibers, χ_w increased slightly with W_t/W_o . This indicates that the enzymatic degradation proceeds from the surface of the drawn fibers and the fine structure of remained inside of fiber is not affected.

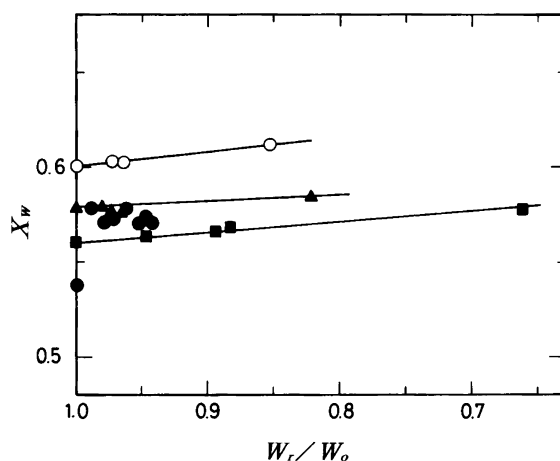


Fig. 9 Change of crystallinity (χ_w) of PCL fibers as a function of weight loss W_r/W_0 with Lipase-PS treatment: (●) DR = 1 (undrawn), (■) DR = 5, (▲) DR = 7, and (○) DR = 9.

Sonic velocity

In order to investigate the effect of molecular orientation on enzymatic degradation, measurements of sonic pulse propagation along fibers before and after Lipase-PS treatments were performed. The speed of the longitudinal wave, C , was determined by measuring the transit time of a sonic pulse. The sonic modulus, E_s , was calculated using the equation

$$E_s = \rho c^2 \quad (3)$$

where ρ is the density of PCL fiber.

The highly drawn PCL fibers show the higher values of E_s due to the increase of crystallinity and the molecular orientation. Figure 10 shows the changes of E_s for undrawn and drawn fibers, which take place during the enzymatic degradation. In the case of undrawn PCL fiber, E_s increased at first and became constant. These changes are due to the change of density with degradation. The changes of E_s

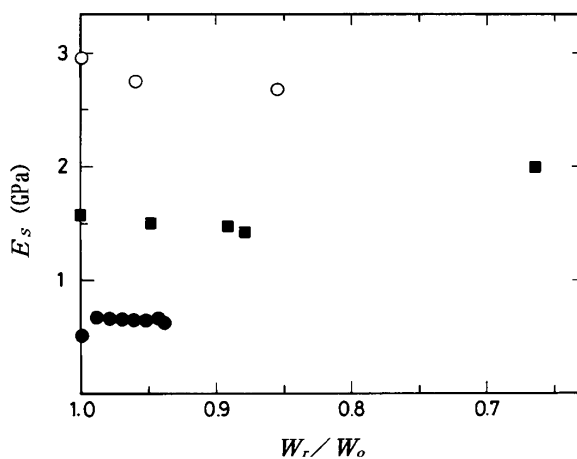


Fig. 10 Sonic modulus E_s of PCL fibers as a function of weight loss W_r/W_0 with Lipase-PS treatment: (●) DR = 1 (undrawn), (■) DR = 5, and (○) DR = 9.

for drawn PCL fibers (DR = 5 and 9) with W_t/W_0 were small at the first stage of degradation. This means that the fine structures of drawn PCL fibers are very stable during the enzymatic degradation. The changes of molecular orientation with W_t/W_0 are negligibly small.

Dynamic viscoelasticity

The structural information for fibers can be obtained from the measurements of temperature dependence of dynamic and loss moduli [13]. Figure 11 shows the curves of temperature dependence of dynamic viscoelastic properties of drawn PCL fiber (DR = 9) before and after treated with Lipase-PS. The α dispersion at about -30°C corresponds to the glass transition of PCL. The investigation of the dynamic mechanical properties of PCL fibers over a wide range of temperature is useful in studying the structure and properties of fibers before and after the enzymatic degradation.

PCL fibers after the Lipase-PS treatment showed macroscopically smooth surfaces. So, the diameter of treated fibers was measured by use of a micrometer and it was used for the calculation of dynamic storage modulus for highly drawn fiber (DR = 9) hardly changed by the treatment. The temperature of $\tan \delta$ peak corresponding to the glass transition of PCL was shifted slightly to the lower temperature.

The dynamic storage modulus E' at -140°C was plotted as the function of W_t/W_0 (Fig. 12). As the mobility of PCL molecular chains is frozen in this temperature region, the dynamic storage modulus depends on the molecular chain orientation of crystalline and amorphous phases. No change of dynamic modulus with the degradation is observed. This means that the average molecular chain orientation does not change during the enzymatic degradation.

In Fig. 13, the dynamic storage moduli E' at 23°C of PCL fibers were plotted as the function of W_t/W_0 . No change of the dynamic modulus means that the enzymatic degradation of PCL fiber takes place from its surface. Figure 14 shows the change of value of $\tan \delta$ at the α dispersion of PCL with W_t/W_0 . This value corresponds to the movement of amorphous phase. The rate of reduction of $\tan \delta$ value of undrawn PCL fiber is at fiber occurred at the first stage of enzymatic degradation as shown above by the result of density change.

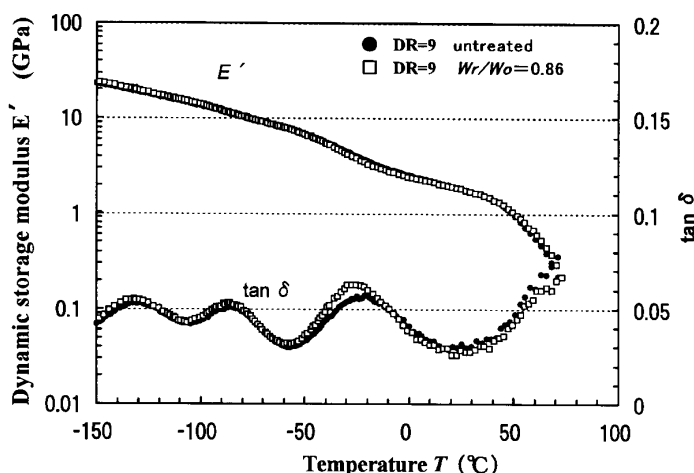


Fig. 11 Temperature dependence of dynamic mechanical properties of drawn PCL fiber (DR = 9) and Lipase-PS treated fiber (DR = 9, $W_t/W_0 = 0.86$). Frequency: 10 Hz.

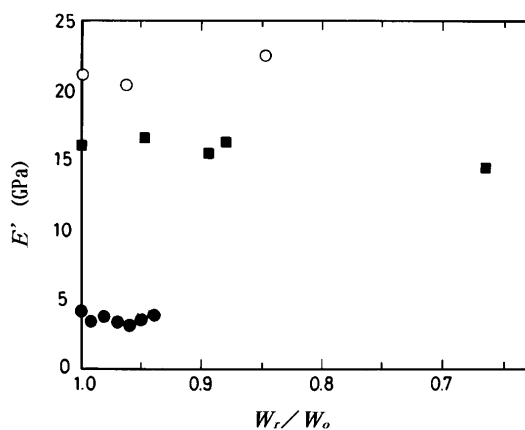


Fig. 12 Dynamic storage modulus E' of PCL fibers at -140 °C as a function of weight loss W_t/W_0 with Lipase-PS treatment: (●) DR = 1 (undrawn), (■) DR = 5, and (○) DR = 9.

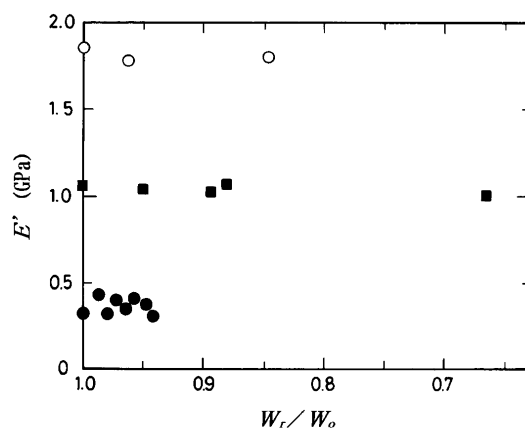


Fig. 13 Dynamic storage modulus E' of PCL fibers at 23 °C as a function of weight loss W_t/W_0 with Lipase-PS treatment: (●) DR = 1 (undrawn), (■) DR = 5, and (○) DR = 9.

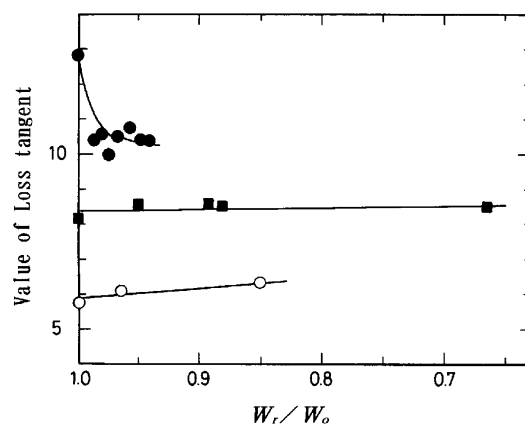


Fig. 14 Change of loss tangent α dispersion peak as a function of weight loss W_t/W_0 with Lipase-PS treatment: (●) DR = 1 (undrawn), (■) DR = 5, and (○) DR = 9.

CONCLUSION

It was suggested from the kinetic study on the weight loss of the PCL fibers with enzymatic degradation in vitro by Lipase-PS that degradation proceeded gradually from the surface of the fiber into the core. Indeed, the observed rate of weight loss was in good agreement with that predicted on the assumption of enzymatic surface erosion. This fact was also supported by SEM, evaluation of crystallinity, and the dynamic viscoelastic properties of degraded PCL fibers. Though the rate of decrease in the tensile strength of the fibers through enzymatic degradation in vitro was higher than that of the weight loss, this feature was quite different from that for the nonenzymatically degradable fibers such as poly(glycolic acid) fiber, which has a rather homogeneous degradation throughout the cross-section of the fiber from the beginning of degradation [16]. The enzymatic degradability was depressed with increasing crystallinity and orientation by drawing. It was concluded that the surface erosion with weight loss by enzymatic degradation appeared to be the primary mechanism similar to the environmental degradation of PCL fibers.

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