Hydrolysis of Polycaprolactone Fibers by Lipase: Effects of Draw Ratio on Enzymatic Degradation

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SYNOPSIS

Using polycaprolactone (PCL) fibers drawn with various draw ratios, the effects of draw ratio on enzymatic degradation were studied in order to understand the influence of fine structure on biodegradation. Degradability of PCL fibers as monitored by total organic carbon (TOC) formation or weight loss decreased with increase in draw ratio due to higher crystalline content. There were some distinct features of degradation behavior among the fibers, because the fibers underwent significant change in molecular organization of the polymer, such as crystallinity and orientation, during drawing processes. From scanning electron microscopy (SEM) photographs showing that the enzyme preferentially attacked amorphous or less ordered regions rather than crystalline or more ordered regions of the fiber, spherulites were observed in the undrawn fiber, and on the other hand, fibrillar stripes along the fiber axis were observed in the drawn fibers, which suggest that the spherulites in the undrawn fiber were extended to be broken, and fibril structures were formed during the drawing processes. These SEM photographs suggest that there are differences of crystal structures in addition to crystallinity among the fibers with different draw ratio, which significantly affects the enzymatic degradation behavior of the fibers. The diameter of the fibers became gradually slim, macroscopically uniform, as enzymatic degradation proceeded although it was dependent on draw ratio. It is evident that the degradation proceeded in the crystalline regions as well as the amorphous regions. © 1995 John Wiley & Sons, Inc.

INTRODUCTION

Recently, environmental problems such as plastic wastes have globally become the center of the public interest. As one of the approaches to solve the problems of plastic wastes, biodegradable polymers that do not pose a hazard to environment have been given attention. Concerning the application of biodegradable polymers for a fiber, bioabsorbable sutures made from polyglycolide, polylactide, or their copolymers, which decompose *in vivo* by chemical hydrolysis, are well known. They are, however, sensitive to moisture, which leads to hydrolysis in the atmosphere, suggesting that they are not suitable for commodity or industrial uses. An environmentally degradable synthetic fiber resistant to moisture and primarily degraded by enzymes secreted by microorganisms in soil or sea water is required for commodity or industrial applications.

Polycaprolactone (PCL) is one of the aliphatic polyesters that is a relatively inexpensive synthetic polymer currently available and truly biodegradable under microbial attack. Degradability of PCL in soil or in sea water,[1,2] by fungi,[3-7] and also by various kinds of enzymes (e.g., lipase) have been reported.[8,9]

The effect of molecular weight, crystallinity, and morphology on the microbial and enzymatic degradation of PCL films have been studied by Huang and his co-workers.[5-7] They have shown that the degradation proceeded in a selective manner, with the amorphous regions being degraded prior to the degradation of the crystalline regions.

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It was found that PCL was melt-spun into fibers with good mechanical properties.[10] We investigated the relationship between noncrystallinity of the PCL fibers and the rate of microbial degradation in field tests, such as soil burial or sea water exposure, to understand the effect of fine structures on biodegradation.[10] The rate of biodegradation in fields, however, depends markedly on the available soil or sea water, which depends on the geographic location.

We report here the enzymatic degradation of PCL fibers having different draw ratios and discuss the effect of draw ratio on enzymatic degradation quantitatively to develop a better understanding of the degradation process.

EXPERIMENTAL

Materials and Preparation of Fibers

TONE P-787 (MI = 4 g/10 min, M_n = 80,000) from Union Carbide Corporation was used. Melt spinning of PCL fibers were carried out at 210°C, followed by drawing at 45°C with 5–9 times draw ratio. To avoid the effect of the surface area on enzymatic degradation, fibers that had almost the same diameters with different draw ratios were manufactured.

Measurements of Tensile Properties

Using an Autograph S-100 (Shimadzu Corporation), tensile properties were measured in accordance with JIS L-1013. A crosshead speed of 30 cm/min and a gauge length of 25 cm was used for the drawn fibers, and a crosshead speed of 10 cm/min and a gauge length of 2 cm was used for the undrawn fiber.

Analysis of Fine Structures

Higher-order structures of PCL fibers were measured by wide-angle X-ray diffraction (WAXD) and small-angle X-ray scattering (SAXS). Both WAXD and SAXS diagrams of the sample were recorded by using CuKα radiation with RAD-rB (Rigaku Denki Co. Ltd.) operated at 50 kV and 200 mA. WAXD profiles were recorded in the range of 2θ = 5–125°. SAXS profiles were recorded along meridian direction of filaments in the range of 2θ = 15–120′, and the meridian curves were evaluated by scattering power index and maximum intensity. Crystallinity was measured by WAXD using the Ruland method. The fiber samples were cut to pieces to eliminate the effect of orientation on the crystallinity. Crystalline orientation was determined by X-ray crystallite orientation measurements from the half-width of azimuthal intensity profile along the ring of (110) plane.

Surface Observations

The surfaces of the fibers were observed by using a field emission type of scanning electron microscopy (SEM), S-4000 (Hitachi Ltd.) with 4-kV acceleration after Au/Pd coating with an ion coater.

Enzymatic Degradation Tests

Each reaction mixture contained 300 mg of PCL fibers, 2.0 mL of 0.2*M* phosphate buffer (pH 7.0), 1.0 mL of 0.1% surfactant Plysurf A210G (Dai-Ichi Kogyo Seiyaku Co. Ltd.), 100 units of *Rhizopus arrhizus* lipase (SIGMA Chemical Company), and distilled water in a total volume of 20 mL. The reaction mixtures were incubated in a 100-mL Erlenmeyer flask on a rotary shaker at 80 rpm at 30°C for 16 h. After the reaction mixtures were filtrated through a millipore filter (0.20 μm), water-soluble total organic carbon (TOC) formation in the reaction mixtures was measured with TOC-500 (Shimadzu Corporation), and weight loss of the fibers were evaluated.

RESULTS

Tensile Properties

Tensile properties of PCL fibers manufactured are shown in Table I. All fibers had almost the same diameters ranging from 272 to 280 μm. Table I gives tensile tenacity of the PCL fibers, which increased with increasing draw ratio, whereas the tendency of ultimate elongation was contrary to it. After enzymatic degradation, both tenacity and ultimate elongation of all fibers decreased drastically and the fibers turned brittle.

Fine Structures

WAXD data of PCL fibers was shown in Table II. A crystallite size along the fiber axis would appear to increase, and crystallite sizes perpendicular to the fiber axis to decrease, and crystal volume to decrease with increasing draw ratio, while crystallinity had tendency to increase. These indicate that crystalline regions increased by drawing although crystallite sizes became smaller.

Table I Tensile Properties of PCL Fibers Prepared

Draw Ratio	Denier (d)	Diameter (μm)	Strength (kg)	Tensile Tenacity (g/d)	Ultimate Elongation (%)
DR = 1 (undrawn)	692	279	0.73	1.05	2485.4
DR = 5 (drawn)	633	272	2.94	4.65	126.0
DR = 6 (drawn)	653	272	3.20	4.90	81.5
DR = 7 (drawn)	675	278	3.52	5.22	58.6
DR = 8 (drawn)	671	280	3.86	5.75	33.3
DR = 9 (drawn)	660	277	4.72	7.15	20.9

Table III shows SAXS data of PCL fibers. Maximum intensity and scattering power index of the original PCL fibers decreased as draw ratio becomes higher. This suggests that difference in electronic density between crystalline and amorphous regions decreased upon drawing, which led the fibers to be highly oriented and highly crystalline.

After enzymatic degradation, crystal volume and crystallinity in all PCL fibers decreased, which indicated that crystalline regions were degraded. In the course of degradation, maximum and scattering intensity of SAXS had a tendency to decrease in the undrawn fiber and drawn fibers whose draw ratio was lower than 5 times, while the opposite tendency in highly drawn fibers was observed. These indicate that the difference in density between crystalline and amorphous regions decreased in undrawn and low drawn fibers, while increasing in highly drawn fibers.

Enzymatic Degradation

Figure 1 shows the amount of TOC products in the reaction mixtures which originated from water-soluble organic compounds, i.e., monomers or oligomers of PCL polymers, after enzymatic degradation. It was found that TOC values decreased with increase in draw ratio, that is, the degradability of PCL fibers was inversely proportional to draw ratio. Figure 2 indicates plots of the weight loss after degradation as a function of draw ratio, showing a similar profile to TOC values with regard to draw ratio.

SEM Observation

Figures 3 and 4 show SEM photographs of the samples with different draw ratios before and after enzymatic degradation. Apparently the diameters of all fibers became smaller uniformly as degradation proceeded, although the higher the draw ratio, the slower the rate of thinning.

Figure 5 shows enlarged SEM photographs of the surfaces of the samples after enzymatic reaction. These SEM photographs indicate the different postdegradation features of the surfaces between the undrawn and drawn fibers. Crystalline spherulites in relief were observed in the undrawn fiber, whereas

Table II Fine Structures of PCL Fibers before and after Enzymatic Degradation Measured by Wide-Angle X-ray Diffractometer

Sample		Crystallite Sizes (Å)			Crystal Volume	Crystallinity	Orientation
Draw Ratio	Degradation	L(110)	L(200)	L(0014)	(Å^3)	(%)	(%)
DR = 1 (undrawn)	Before	79	74	61	357,000	40.0	65.3
	After	73	62	57	258,000	38.7	63.3
DR = 5 (drawn)	Before	74	64	62	294,000	63.3	94.4
	After	81	59	60	287,000	57.3	95.3
DR = 7 (drawn)	Before	73	53	66	255,000	65.0	95.3
	After	76	54	50	207,000	61.8	95.1
DR = 9 (drawn)	Before	68	49	68	227,000	69.3	95.7
	After	73	48	59	205,000	63.9	95.3

Table III Small-Angle X-ray Scattering of PCL Fibers before and after Enzymatic Degradation

Sample		Maximum Intensity (cps)	Long Period (Å)	Scattering Power Index
Draw Ratio	Degradation			
DR = 1 (undrawn)	Before	3969	179	846,000
	After	3743	180	724,000
DR = 5 (drawn)	Before	1991	164	473,000
	After	1832	165	438,000
DR = 7 (drawn)	Before	841	165	190,000
	After	930	167	213,000
DR = 9 (drawn)	Before	579	193	95,000
	After	618	200	108,000

fibrillar stripes in relief along the fiber axis were observed in the drawn fibers.

DISCUSSION

Crystalline parameters, such as crystallinity or crystalline orientation, of PCL fibers increased rapidly with draw ratio and then reached a plateau at draw ratios of around 5. However, the tensile strength began to increase significantly after these crystalline parameters began to level off, reaching over 7 g/d at 9 times drawn. It is a well-known feature characteristic to crystalline polymers.

The crystalline structures mainly consisting of extended chain crystals developed in PCL fibers during drawing processes since PCL is a crystalline polymer, which account for the fact that crystallinity and crystalline orientation increased with increasing draw ratio. It is also considered that tenacity increases due to an increase of extended chain crystals and tie molecules in the amorphous regions between the crystals.

From the results of TOC value and weight loss, it was found that the rate of enzymatic breakdown of PCL fibers decreased with increase in draw ratio. It is supposed that enzymatic degradation of PCL proceeds preferentially in the amorphous regions having sufficient spatial degree of freedom that enzyme can be physically adjacent to polymer molecule and penetrate into the polymer.[7,11] Therefore, it is considered that degradability is smaller in the highly drawn fibers that have more crystalline regions and highly oriented amorphous regions than in the undrawn fibers that have less crystalline regions with lower orientation. It is the reason why the degradability of PCL fibers was inversely proportional to draw ratio. It is suggested that the crystalline regions of PCL fibers also can be ultimately degraded as the amorphous regions are degraded, corresponding to

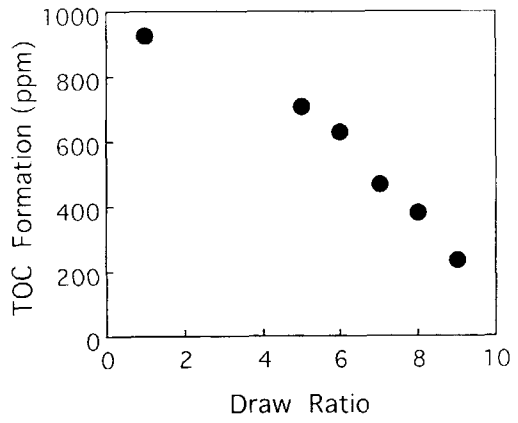

Figure 1 TOC formation of PCL fibers with different draw ratio after 16 h in the aqueous solution containing lipase of *Rhizopus arrhizus* at 30°C.

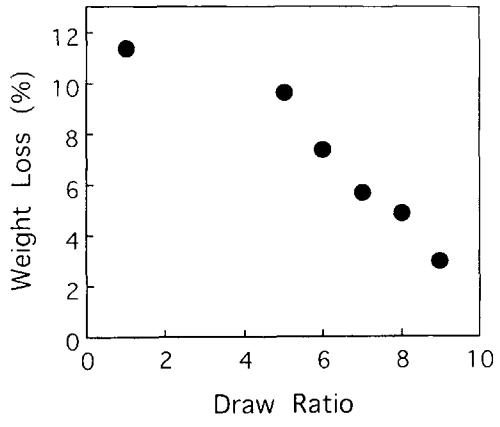

Figure 2 Weight loss of PCL fibers with different draw ratio after 16 h in the aqueous solution containing lipase of *Rhizopus arrhizus* at 30°C.

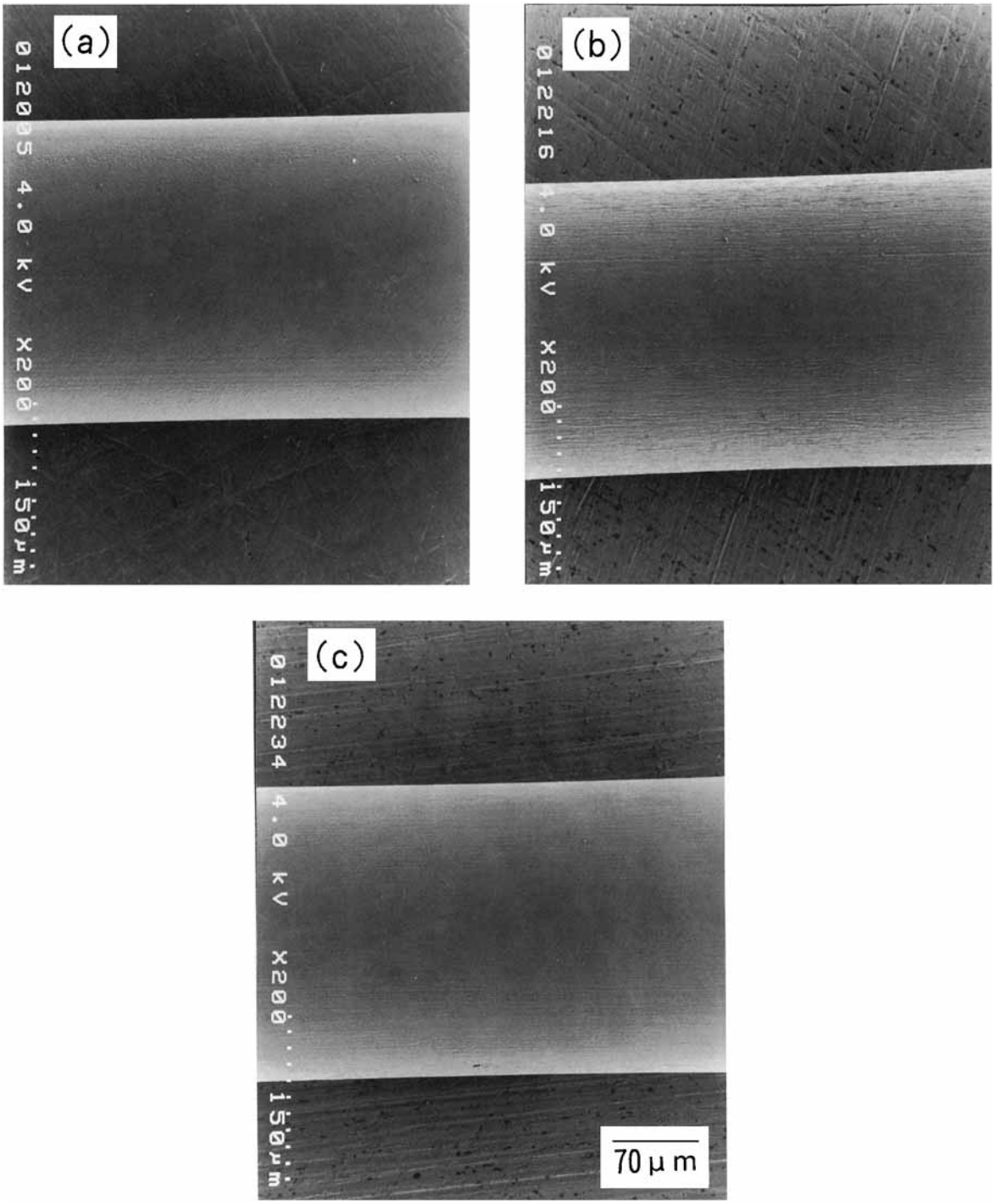

Figure 3 SEM photographs of PCL fibers before enzymatic degradation. (a) DR = 1 (undrawn); (b) DR = 5 (drawn); (c) DR = 9 (drawn).

the fact that the diameter of the fibers became uniformly thin with the progress of degradation.

SAXS data indicate that there are differences of degradable behaviors in PCL fibers with different draw ratios. The observed SAXS is due to heterogeneities of electronic density in the dimensional range of 20–300 Å, which originated from two distinct intermixed microphases representing the crystalline and noncrystalline regions, respectively. After exposure to enzyme in liquid medium, the differences

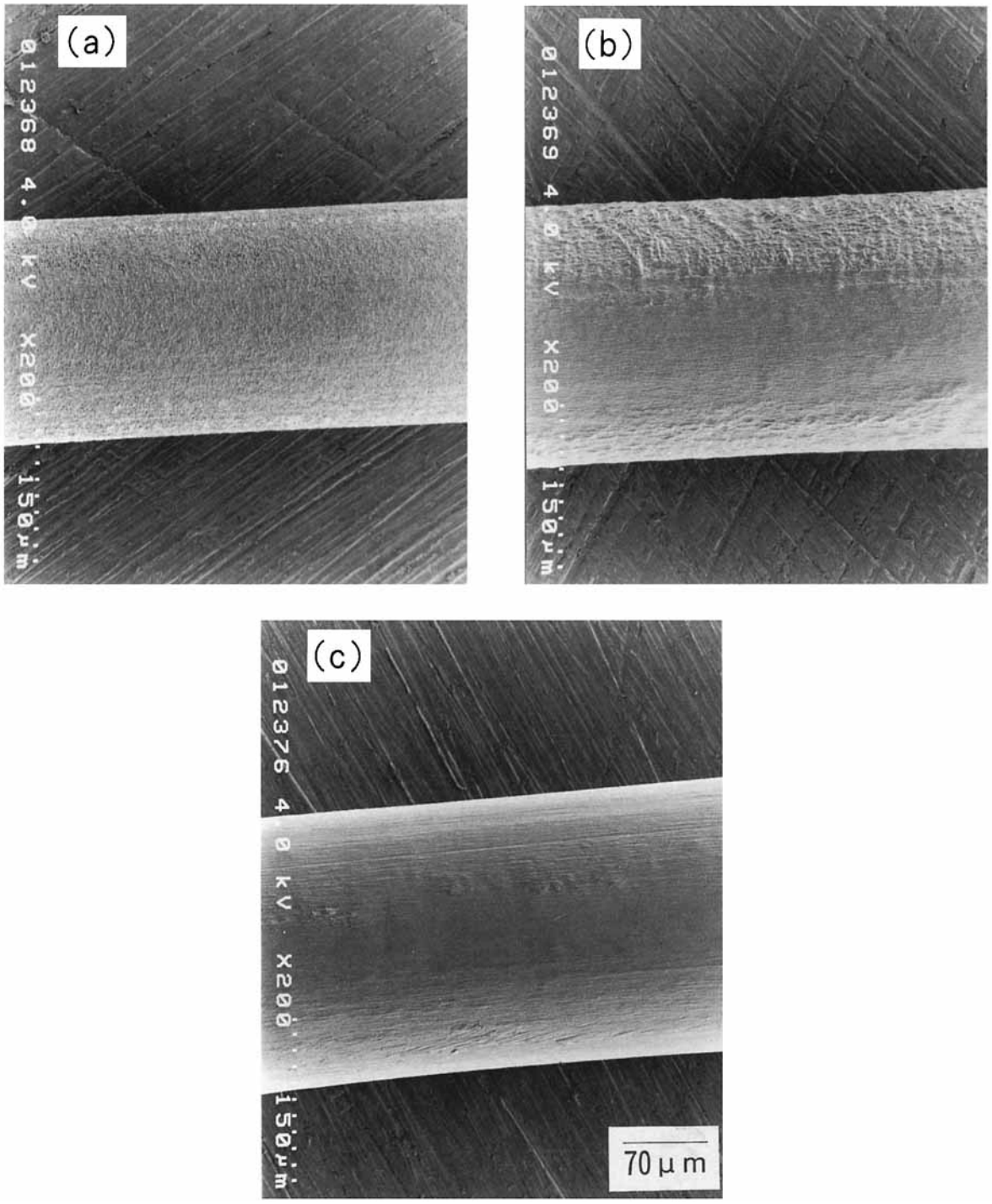

Figure 4 SEM photographs of PCL fibers after enzymatic degradation. (a) DR = 1 (undrawn); (b) DR = 5 (drawn); (c) DR = 9 (drawn).

in electronic density between crystalline and amorphous components of the undrawn or less drawn fibers decreased, whereas that of the highly drawn fibers increased. In the former case, it would be due to preferential erosive degradation in the loose amorphous regions, which are dominant in the undrawn or less drawn fibers. In contrast, in the latter case, it is reasonable to assume that the density of amorphous regions became lower by enzymatic degradation due to disorientation of highly oriented re-

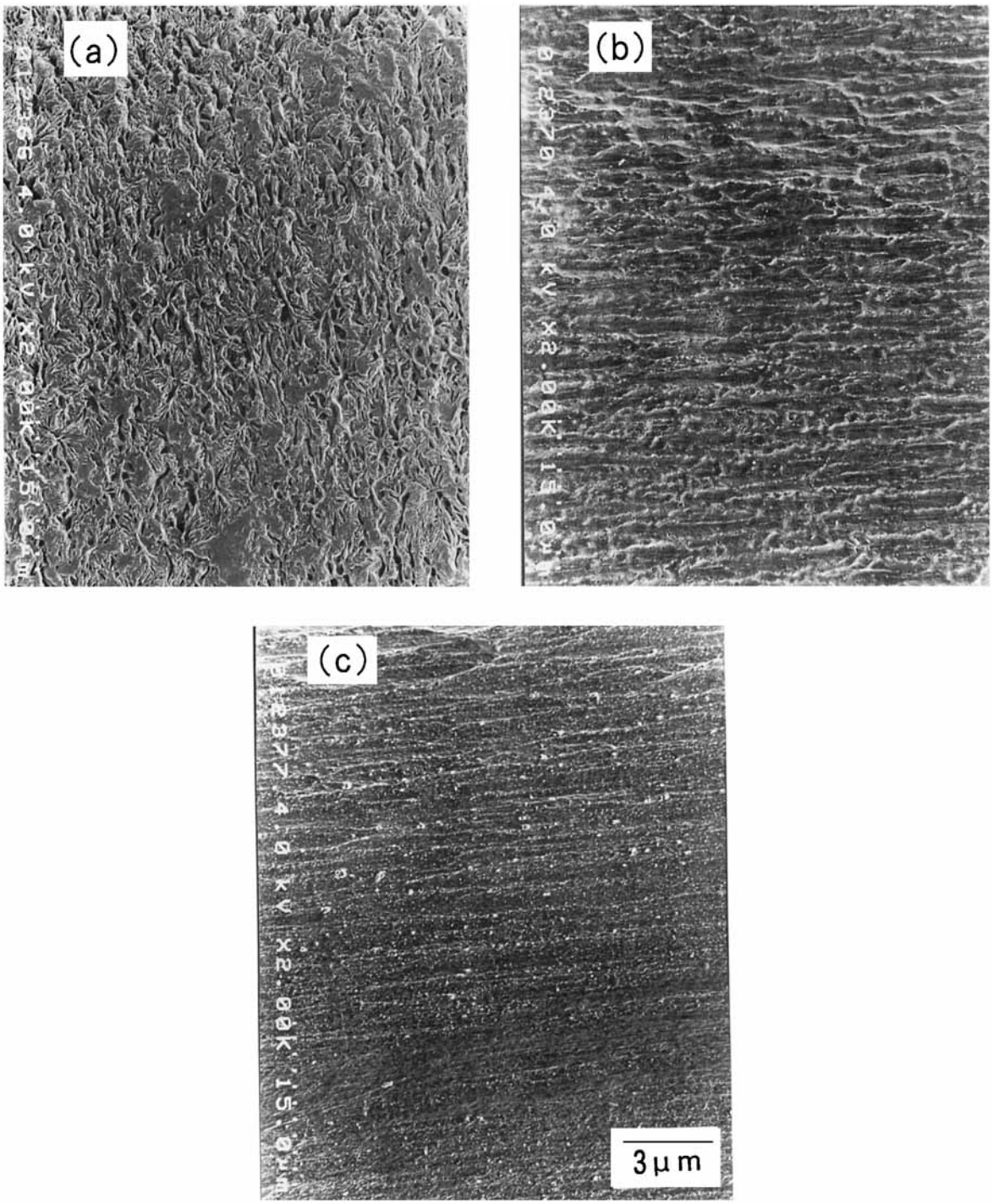

Figure 5 SEM photographs of PCL fibers undrawn and drawn in horizontal direction after enzymatic degradation. (a) DR = 1 (undrawn); (b) DR = 5 (drawn); (c) DR = 9 (drawn).

gions dominant in the amorphous regions of the highly drawn fibers, which lead to the fact that the differences in electronic density between crystalline and amorphous components became bigger.

SEM observations indicate that enzymatic degradation in the undrawn fibers proceeds from the surface to the inside of the fibers accompanied with a decrease in the diameter of the fibers. The lipase

studied here is an endoenzyme that is responsible for the enzymatic degradation of the amorphous areas of the spherulites prior to the degradation of the crystalline areas. The initial stage of enzymatic degradation may be a random scission of the in-chain ester linkage in the amorphous regions, resulting in selective erosion of the amorphous regions, which is probably necessary for further endoenzyme attack on the crystalline regions. Then the exposed spherulites were also subsequently degraded. In contrast, there could be seen no spherulites but fibrillar stripes parallel to the fiber axis in the drawn fibers, suggesting that spherulites in the undrawn fibers were extended to be broken, i.e., unfolding of lamellar crystals, during drawing processes, and fibril structures were formed. These stripes became finer as draw ratio became higher due to an increase of orientation of the fibrils and interfibrillar extended amorphous chain segments, as seen in Figure 5(b) and 5(c). Degradation in the drawn fibers first occurred in the amorphous regions between oriented fibrils along the fiber axis, followed by degradation of the fibrils themselves. The diameters of the fibers became smaller uniformly as the degradation proceeded in the same way as in the undrawn fibers, except markedly slower. The differences of morphology caused by drawing account for the different degradation behaviors.

CONCLUSION

From SEM observations combined with analysis of fine structure, the spherulites of undrawn fiber were broken to result in the fibril structures with increased crystallinity during drawing. The rate of enzymatic degradability of the fibers as monitored by TOC formation or weight loss is faster in the case of lower draw ratio than in the case of higher draw ratio. The faster rates of degradation at lower draw ratio might be attributed to less crystallinity. First, degradation appears to proceed preferentially in the less ordered amorphous regions, and then the crystalline regions were ultimately degraded also as the amorphous regions were degraded, leading the diameter of the fiber to become uniformly smaller and smaller with exposure time.

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