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Enzymatic degradation of bacterial homo-poly(3-hydroxybutyrate) melt spun fibers

H. Yamane[a,*], K. Terao[a], S. Hiki[b], Y. Kawahara[a], Y. Kimura[b], T. Saito[c]

[a]*Division of Advanced Fibro-Science, Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 606-8585, Japan*
[b]*Department of Polymer Science and Engineering, Kyoto Institute of Technology, Kyoto 606-8585, Japan*
[c]*Department of Science, Kanagawa University, Kanagawa, Hiratsuka 524-1293, Japan*

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Abstract

The effect of the higher order structure on the degradation behavior of bacterial homo-poly(3-hydroxybutyrate) (PHB) melt spun fibers by a PHB depolymerase from *Commamonas testosteroni* was investigated. After the enzymatic degradation, fibrillar structure remained at the surface of the drawn and annealed fibers, while a spongy structure appeared in the as-spun fiber. Similar spongy structure to the degraded as-spun fiber was observed in the core part of some drawn and annealed fibers after the fibrillar surface disappeared. The mechanical properties deteriorated as enzymatic degradation proceeded. After the amorphous region was degraded, β-form crystal, which seems to have less ordered structure, degraded in advance to the α-form crystal as revealed by WAXS measurements. However, these tendencies were less pronounced for the fiber annealed at high temperature under high tension. This result suggests that the β-form crystal in the fiber annealed at high temperature under high tension would have more ordered crystalline structure, which withstands against the attack by the enzyme.

Keywords: Bacterial homo-poly(3-hydroxybutyrate); Melt spun fibers; PHB depolymerase

1. Introduction

Bacterial poly(3-hydroxybutyrate) (PHB) is produced by various microorganisms as an energy storage product [1]. This polymer is one of the biodegradable polymers which can degrade completely in the environment without forming any toxic products. There are a lot of reports on the biodegradation of PHB in soil, sludge, sea water and buffer solutions containing various PHB depolymerases [2–11]. However, most studies reported were carried out on PHB granules and solution cast films and the enzymatic degradation behavior of processed PHB into fibers has not been reported.

Kumagai et al. [4] reported that the rate of enzymatic degradation of PHB film by a PHB depolymerase from *Alcaligenes facalis* is strongly influenced by the degree of crystallinity, although it is less dependent on the size of spherulites, which is against the results obtained by Tomoshi et al. [9]. Tomoshi et al. [9], utilizing the PHB films isothermally crystallized at various temperatures, reported that the rate of enzymatic hydrolysis of PHB film decreases with increasing crystal size when the crystallinity of the films were similar. Iwata et al. [10] demonstrated that the attack by the active site of PHB depolymerase takes place preferentially at the disordered chain packing region of crystal edge rather than the chain-folding surfaces of single crystal.

Various efforts have been paid to process PHB and PHB copolymers into fibers, films and other products. Furuhashi et al. [12,13] and Yamamoto et al. [14] succeeded the melt spinning and cold drawing of poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) and reported their mechanical properties and detailed higher order structure. Yamane et al. [15] also succeeded the melt spinning and drawing of roughly purified bacterial homo-PHB and reported the mechanical properties and the higher order structure of resultant fibers. Recently, Schmack et al. [16] reported the effect of high-speed melt spinning and spin-drawing on the higher order structure and resulting properties of bacterial homo-PHB.

In the present study, we were concerned with the effect of higher order structure of melt spun bacterial homo-PHB fibers obtained in various drawing and annealing conditions [15] on the enzymatic degradation behavior.

* Corresponding author. Tel.: +81-75-724-7824; fax: +81-75-724-7337.
E-mail address: hyamane@ipc.kit.ac.jp (H. Yamane).

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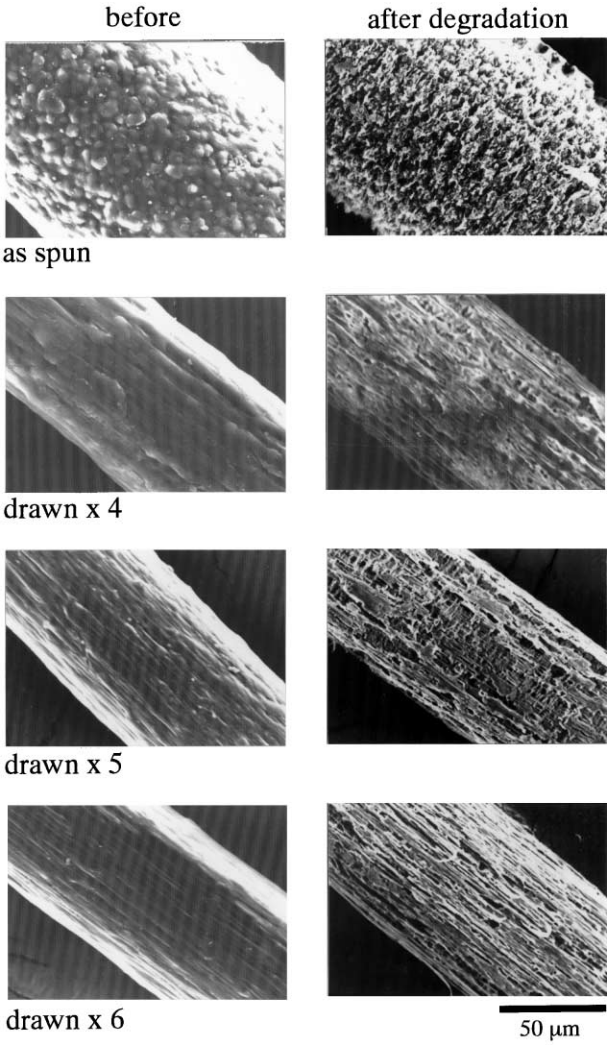

Fig. 1. SEM photographs of as-spun and drawn PHB fibers before and after 24 h of enzymatic degradation.

2. Experimental

2.1. Materials

Bacterial homo-PHB was supplied by Monsanto, Japan, and used without further purification. Sample was dried in vacuo at 80°C for 10 h before melt spinning.

2.2. Preparation of PHB fibers

Melt spinning of PHB was carried out using a laboratory-size screw extruder equipped with a single nozzle with 1 mm in inner diameter. PHB was extruded at the melting zone temperature of 180°C and the nozzle temperature of 170°C. The extrudate was taken up at 28 m/min and directly drawn to various ratios using a drawing machine with two sets of rolls and a hot plate heated at 110°C.

Annealing of drawn fibers drawn to six times was conducted at various temperatures and under various tensions. The PHB fiber was passed through the air, heated at 100, 125 and 150°C under the tensions of 0, 50 and 100 MPa.

Thermal and mechanical properties and the higher order structure of these fibers are described in the previous report [15].

2.3. Enzymatic degradation

The segments of fibers cut into 6 cm were placed in test tubes containing 10 ml of potassium phosphate buffer (pH 7.4) and 136 units of PHA depolymerase from *Commamonas testosteroni* [11]. The reaction mixtures were incubated at 37°C. After the predetermined period, segments of fibers were taken out, washed three times with distilled water, and dried in vacuo for 24 h. Extent of enzymatic degradation was determined by the tensile tests, SEM observations and WAXS measurements.

2.4. Wide angle X-ray scattering

WAXS diffractometer scans were obtained using a CuKα radiation (40 kV, 30 mA) with RINT 2100 FSL (Rigakudenki).

2.5. Mechanical properties

Mechanical properties of the fibers were evaluated by the tensile tests with a tensile testing machine (CATY-500BH: Yonekura). A specimen gauge length of 20 mm was used. The tests were carried out at a cross-head speed of 50 mm/min at a room temperature. The results obtained were averaged over five samples for each condition. Diameters of the fibers before and at an initial stage of enzymatic degradation were measured with a thickness gauge and those of the fibers fairly degraded were measured with an optical microscope. Even though the tensile tests were possible for the fibers seriously degraded, diameter measurements were not possible and the data obtained were omitted from the discussion.

2.6. Morphology

Scanning electron micrographs (SEM) were taken by using a JSM-25S (Jeol) microscope at an electron voltage of 5–20 kV after samples were subjected to Au coating.

3. Results and discussion

3.1. Change in the morphology of drawn PHB fibers during enzymatic degradation

Fig. 1 shows SEM photographs of as-spun and drawn PHB fibers before and after 24 h of enzymatic degradation. The surface of as-spun PHB fiber before degradation indicated the presence of many large spherulites due to the crystallization, gradually occurred after melt spinning.

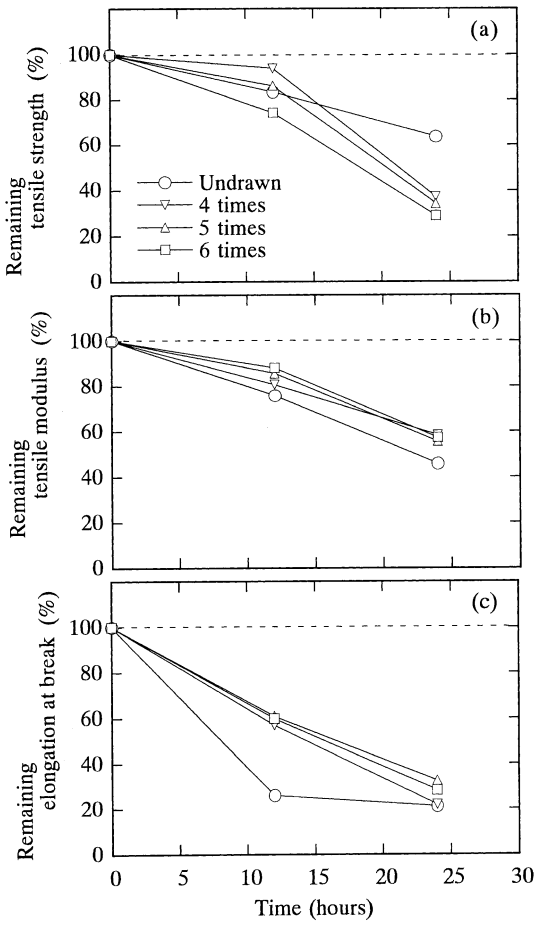

Fig. 2. Percent remaining in the mechanical properties: (a) tensile strength; (b) tensile modulus; (c) elongation at break, of as-spun and drawn PHB fibers during enzymatic degradation.

Drawn fibers before degradation have fairly rough surface. The fiber drawn to 6 times has fibrillar structure. The photograph of as-spun fiber after degradation shows that the degradation started at the less ordered region around the spherulitic crystals. In addition, it can be seen that the fiber diameter decreases since the degradation started at the fiber surfaces and proceeded to the core region. Similarly the drawn fibers also degraded at the less ordered region remaining the fibrillar structure. Particular difference in morphology among the fibers drawn to various ratios was not clearly observed. After 4 days of enzymatic degradation, the drawn fiber became the aggregate of small fibrous fragments, while as-spun fiber still kept its fiber shape with spongy structure although the diameter significantly decreased. This difference can be attributable to the difference in the surface area of these fibers. Undrawn fiber has similar degree of crystal to that of drawn fiber [15]. If the structure of as-spun and drawn fibers is almost identical, the thinner drawn fibers degrade more rapidly. Besides, the drawn fibers consist of fibrillar crystals. These fibrillar crystals are not inter-connected to each other and the fiber can be broken into very thin fibrils.

3.2. Change in the mechanical properties of drawn PHB fibers during enzymatic degradation

Enzymatic degradation of the drawn and as-spun fibers proceeded rapidly so that the tensile tests were no longer possible after more than 24 h of degradation. Even for the tensile tests of the fibers degraded in a shorter period of time, a special care was necessary.

Percent remaining in the mechanical properties of as-spun and drawn fibers during enzymatic degradation are shown in Fig. 2(a)–(c). The tensile strength of both as-spun and drawn fibers decreased monotonically with time. Since as-spun fiber is brittle and has very low strength even before degradation, change in the strength is not obvious. Deterioration of the strength proceeded more rapidly with increasing draw ratio. This is due to the smaller diameter of highly drawn fibers. On the other hand, the tensile modulus of as-spun fiber decreased slightly faster than drawn fibers. The elongation at break of as-spun fiber suddenly decreased in 6 h and then stayed almost constant, while that of drawn fibers decreased linearly with time irrespective of draw ratio.

3.3. Change in the morphology of annealed PHB fibers during enzymatic degradation

Annealing was carried out only to the fiber drawn to six times. Figs. 3 and 4 show the SEM photographs of annealed PHB fibers after 24 and 48 h of enzymatic degradation, respectively. The degradation of the fibers annealed without tension progressed more rapidly than unannealed fiber while the fibers annealed under tension degraded more slowly than unannealed fiber. When the tension was not applied, the effect of the annealing temperature was not very clear. This is probably due to the competitive effects between the relaxation of the molecular orientation and crystallinity enhancement. Annealing at a high temperature tends to randomize molecular orientation while it increases the crystallinity.

The fibers annealed without tension started losing the fibrillar surface in 24 h, and only the core part, which is composed of a structure similar to that of degraded as-spun fiber, remained even after 48 h of degradation. Similar structure in a core region was also observed in the fibers annealed under 50 MPa in tension. This suggests that only the surface area is highly oriented and the core part is composed of the spherulitic structure similar to that of as-spun fiber. This sort of structure was not clearly observed in the fibers annealed under 100 MPa in tension and the fibers still keep fibrillar surface even after 48 h of degradation.

3.4. Change in the mechanical properties of PHB annealed fibers during enzymatic degradation

Degradation of the unannealed fiber and the fiber annealed at 100°C without tension proceeded rapidly so that the tensile tests were no longer possible over 24 h.

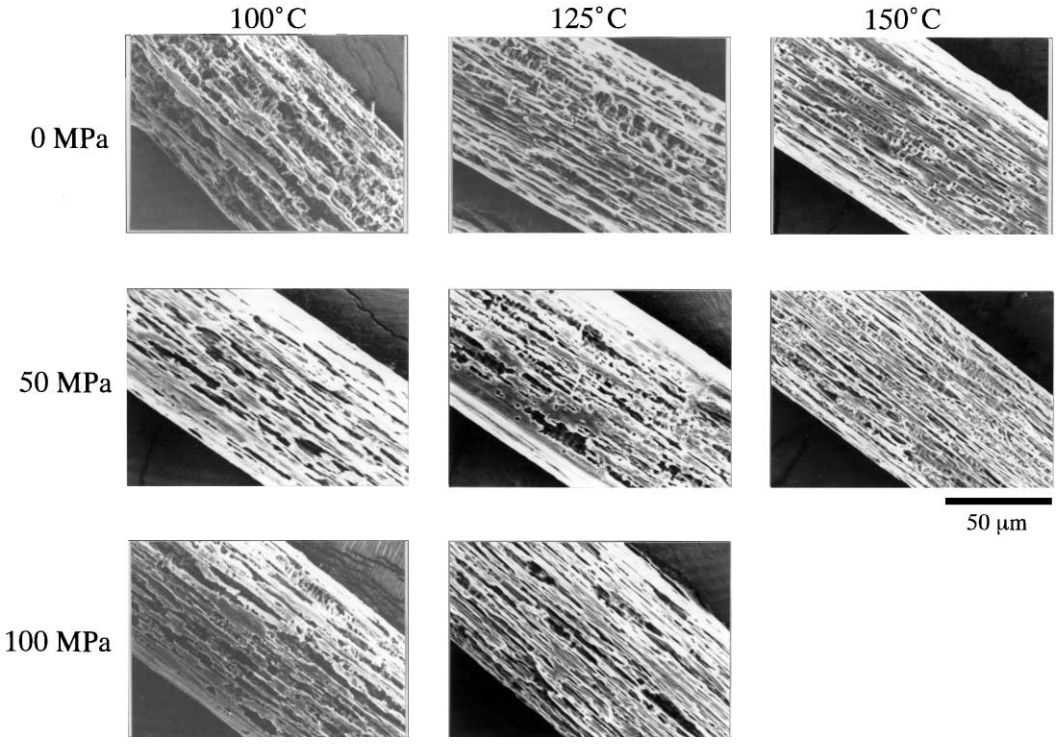

Fig. 3. SEM photographs of annealed (drawn to six times) PHB fibers after 24 h of enzymatic degradation.

The fibers annealed at higher temperatures under 100 MPa in tension kept their strength much longer.

Change in the tensile strength of the fibers annealed without tension and under 100 MPa in tension during enzymatic degradation are compared in Fig. 5(a) and (b), respectively. Tensile strength decreased monotonically with time. The fiber annealed at 125°C under 100 MPa in tension, which has the highest strength before degradation, showed the

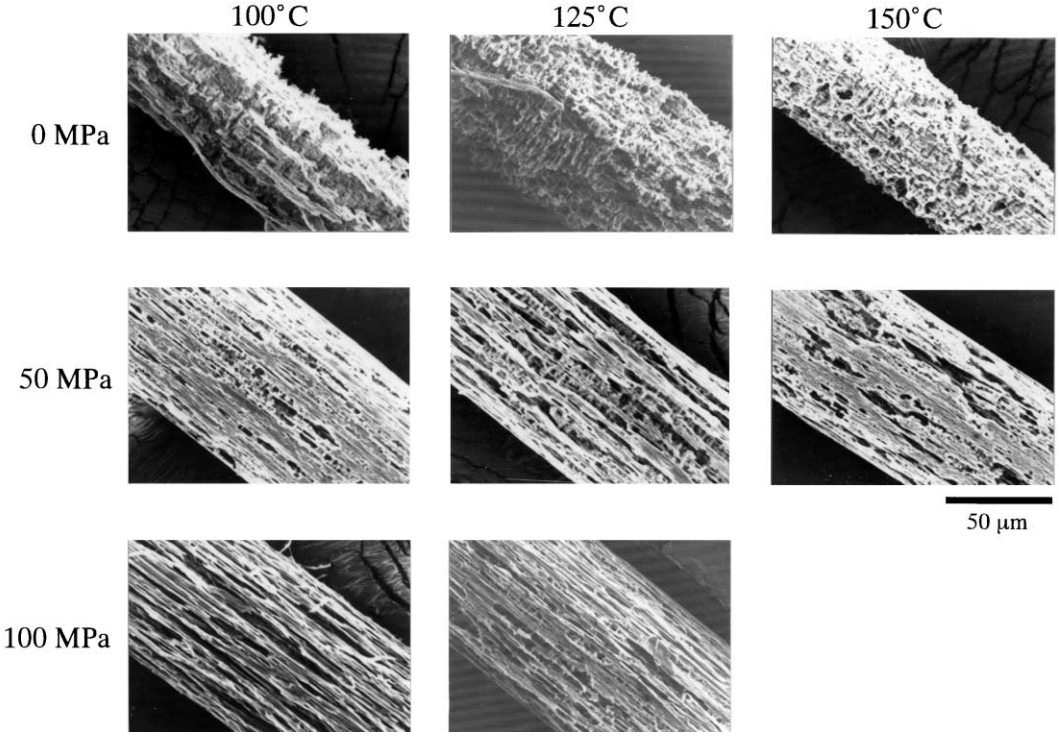

Fig. 4. SEM photographs of annealed (drawn to six times) PHB fibers after 48 h of enzymatic degradation.

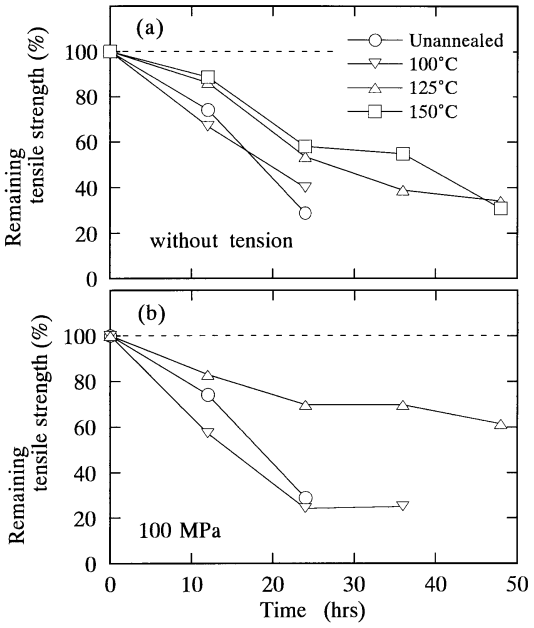

Fig. 5. Percent remaining in the tensile strength of PHB fibers annealed (a) without and (b) under 100 MPa in tension (drawn to six times) during enzymatic degradation.

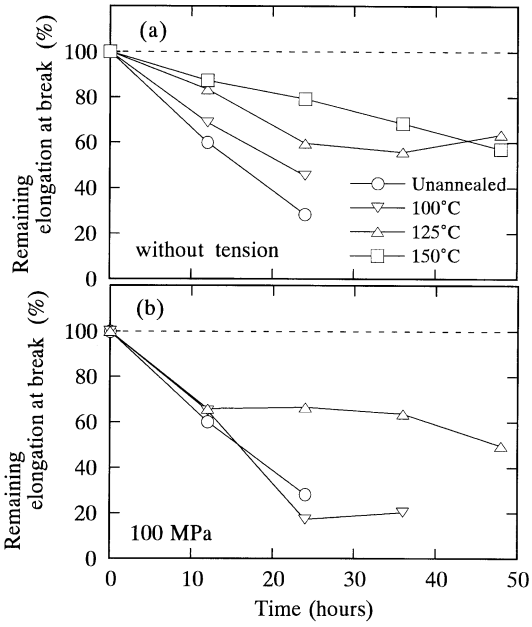

Fig. 7. Percent remaining in the elongation at break of PHB fibers annealed (a) without and (b) under 100 MPa in tension (drawn to six times) during enzymatic degradation.

slowest deterioration rate among all fibers examined. This result is easily expected from the morphological studies described in the previous section.

Change in the tensile modulus of the fibers annealed without and under 100 MPa in tension during enzymatic degradation are shown in Fig. 6(a) and (b), respectively. Modulus of all the fibers decreased with time except for the fiber annealed at 125°C under 100 MPa in tension. This fiber did not show a decrease in modulus within the time period examined and even showed a higher modulus. This may be attributable to the loss of the amorphous parts which generally give a lower modulus.

Very similar trend was also observed for the elongation at break as seen in Fig. 7(a) and (b), where the fiber annealed at 125°C under 100 MPa in tension showed much smaller decrease than other fibers.

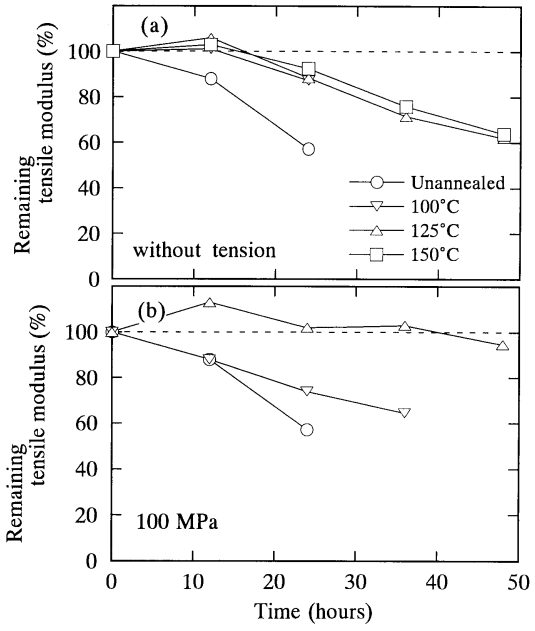

Fig. 6. Percent remaining in the tensile modulus of PHB fibers annealed (a) without and (b) under 100 MPa in tension (drawn to six times) during enzymatic degradation.

3.5. Change in the crystalline structure during enzymatic degradation

PHB fibers prepared in this study are composed of α- and β-forms of crystalline region as well as amorphous region. Formation of β-form induced by the cold drawing was demonstrated by Orts et al. [17] who suggested that the β-form chains emanate upon stretching from the amorphous domains between orthorhombic α-form lamellae. Our previous report [15] showed that β-form is produced during drawing and annealing processes and is rather disordered than α-form as revealed by DSC measurements and WAXS studies.

Fig. 8 shows the equatorial WAXS scans of unannealed (drawn up to 6 times) PHB fibers before and after 36 h of enzymatic degradation. Some of the curves in Figs. 8 and 9 are shifted horizontally to avoid overlapping. The peaks observed at $2\theta = 13.4$ and 16.0°, assigned to be the reflections from α-form stayed unchanged during degradation, while that observed at $2\theta = 20$°which is assigned to be a reflection from β-form, almost disappeared after degradation. Similar results were obtained for the fibers annealed at 125°C without tension as shown in Fig. 9. Iwata et al. [10]

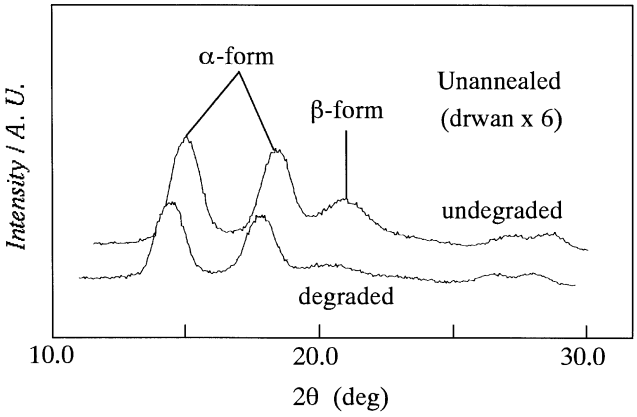

Fig. 8. Equatorial WAXS scans of unannealed (drawn to six times) PHB fiber before and after 36 h of enzymatic degradation.

reported that the attack by the PHB depolymerase takes place preferentially at the disordered chain packing region of crystal edge. Their report may support our results that the disordered β-form is attacked by the depolymerase more rapidly than more ordered α-form. On the other hand, the intensity of the peak at $2\theta = 20^\circ$ observed for the fiber annealed at 125°C under 100 MPa in tension did not change significantly during degradation. These results indicated that the β-form of the crystal formed during cold drawing and annealing without tension is rather disordered, while that formed by annealing process under high tension is more ordered and resists against to the attack by the PHB depolymerase.

4. Conclusion

The effect of the higher order structure on the enzymatic degradation behavior of bacterial homo-PHB melt spun fibers was investigated. After the enzymatic degradation, fibrillar structure remained at the surface of the drawn and annealed fibers, while a spongy structure appeared in the as-spun fiber. Similar spongy structure to that of the degraded as-spun fiber was observed in the core part of the drawn and annealed fibers after the fibrillar surface was disappeared. The mechanical properties deteriorated as enzymatic degradation proceeded. After the amorphous region was degraded, β-form crystal, which seems to have less ordered structure, degrade in advance to the α-form crystal as revealed by WAXS measurements. However, this tendency was less pronounced for the fiber annealed at high temperature under high tension. This result suggests that the β-form crystal in the fiber annealed at high temperature under high tension would have more ordered crystalline structure.

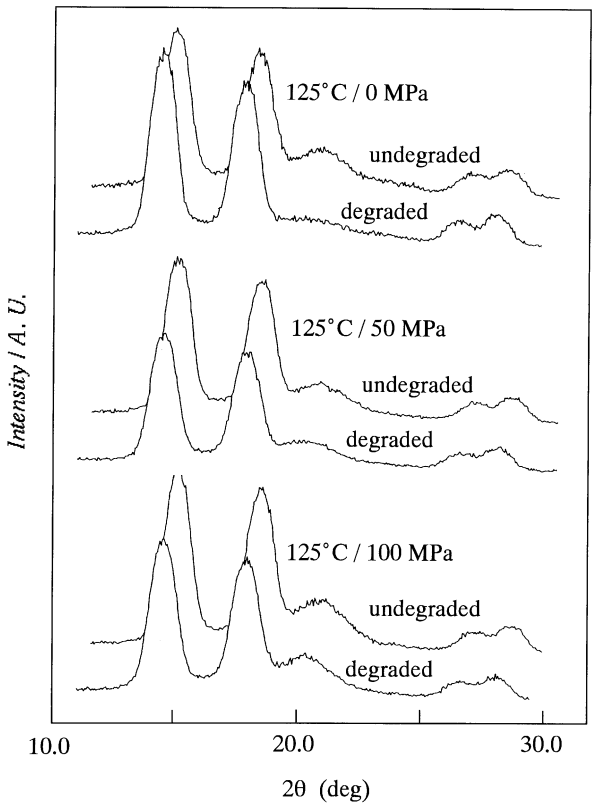

Fig. 9. Equatorial WAXS scans of PHB fibers annealed at 125°C under various tension before and after 36 h of enzymatic degradation.

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