Control of Biodegradability of Poly(3-hydroxybutyric acid) Film with Grafting Acrylic Acid and Thermal Remolding

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ABSTRACT: Radiation-induced graft polymerization of acrylic acid (AAc) on poly(3-hydroxybutyric acid) (PHB) film was carried out and the resulting film was thermally-remolded. The PHB films grafted with AAc (PHB-*g*-AAc) having a degree of grafting higher than 5% completely lost the enzymatic degradability. The enzymatic degradability of the grafted film was recovered by thermal remolding. The highest enzymatic degradation rate was observed at degree of grafting of 10% after thermal remolding. The PHB-*g*-AAc films and thermally-remolded PHB-*g*-AAc films were characterized by contact angle and differential scanning calorimetry. The enzymatic degradability of PHB-*g*-AAc films was lost by the grafted AAc, which covered the surface of PHB film. The acceleration of enzymatic degradation in the remolded PHB-*g*-AAc films was mainly caused by decrease of crystallinity of PHB by dispread of grafted AAc during thermal remolding. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 101: 3856–3861, 2006

Key words: poly(3-hydroxybutyric acid); radiation graft; enzymatic degradation; crystallization

INTRODUCTION

Poly(3-hydroxybutyric acid) (PHB) and its copolymers, poly(hydroxyalkanoic acid)s (PHA), are thermoplastic polyesters produced by bacteria such as *Ralstonia eutropha*, *Alcaligenes latus*, and *Delftia acidovorans*.[1–3] These PHA polymers have many advantages such as biodegradability and biocompatibility. These degradable PHA polymers have attracted much attention to be used for a wide range of agricultural, industrial, and medical applications.[4] However, PHB has several inherent deficiencies such as brittleness due to its high crystallinity and thermal instability.

To overcome such demerits of PHB, many approaches have been adopted. One of the approach is to biosynthesize copolymers containing PHA units. For example, poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) [P(3HB-*co*-3HV)],[5] poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) [P(3HB-*co*-4HB)],[6] and poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) [P(3HB-*co*-3HH)][7] have been biosynthesized by a variety of bacteria with various carbon sources. These copolymers have a wide range of melting points and crystallinity with controlled mechanical properties.

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The second approach is to prepare the blend polymers of PHB and other chemically-synthesized polymers such as poly(ethylene oxide) (PEO),[8–11] poly(vinyl alcohol) (PVA),[12,13] poly(vinyl acetate) (PVAc),[14] poly(vinylidene fluoride) (PVdF),[13] poly(vinyl phenol) (PVPh),[13] poly(methyl methacrylate) (PMMA),[15–17] and polystyrene (PS).[17] The blending of hydrophilic PEO accelerated the enzymatic degradability of PHB.[10] On the other hand, the enzymatic degradation of the blends of P(3HB-*co*-3HV) and PS was found to be suppressed by the hydrophobic PS.[17]

The third approach is to use the radiation grafting onto PHB. In the previous study, the radiation grafting onto PHB powder has been investigated by using acrylic acid (AAc),[18] methyl methacrylate (MMA), 2-hydroxyethyl methacrylate (HEMA),[19] and styrene (St).[20–22] Enzymatic degradability of PHB powder grafted with MMA steeply decreased, whereas enzymatic degradability of PHB increased by introduction of hydrophilic HEMA. From these results, it was concluded that the biodegradability of grafted PHB would be affected by hydrophilicity of grafted polymers. To investigate the effect of graft chains on the enzymatic degradation in detail, AAc was grafted on the PHB films. As a result, the biodegradability of the PHB grafted with AAc (PHB-*g*-AAc) films was considerably reduced with increase of degree of grafting, though hydrophilic polymer was grafted. And then, the enzymatic degradability of these grafted films was recovered by thermal remolding.

We consider that the control of biodegradability of polymer is so important for biodegradable polymer, especially in the agricultural field. Grafting is a promising technique to give the novel biodegradable polymer, which is not biodegraded in use and becomes biodegradable after thermal treatment. In the present article, the irradiation effect of PHB film and the effect of grafting AAc on the biodegradability were investigated before and after thermal remolding. The mechanism of degradability change was clarified.

EXPERIMENTAL

Preparation of poly(3-hydroxybutyric acid) film

Poly(3-hydroxybutyric acid) (PHB) was purchased from Aldrich Chemical. PHB was purified as follows: PHB was dissolved in chloroform and then poured into the mixed solvent of *n*-hexane and methanol (1:1 vol %). The precipitated PHB was isolated by filtration and dried in a vacuum. PHB films with size of 150 mm × 150 mm and 150 μm thick was obtained by hot-press at 190°C for 5 min. The PHB films were crystallized isothermally at 90°C for a week before use.

Acrylic acid (AAc) was purchased from Tokyo Kasei and used without further purification.

Radiation-induced graft polymerization of poly(3-hydroxybutyric acid) with acrylic acid

The PHB grafted with AAc (PHB-*g*-AAc) films were prepared by using preirradiation techniques of radiation-induced graft polymerization. The PHB films were cut into small ones of 10 mm × 60 mm and then packed into polyethylene bags with sealing. After substitution of air in the bags for nitrogen gas, they were irradiated with 2.0 MeV and 1 mA electron beams (EB). These irradiated PHB films were put into glass reactor. Deaerated AAc and methanol (AAc concentration: 5–50 wt %) were sucked into the glass reactor. The grafting temperature was maintained at 40°C by dipping the glass reactors in a warm bath. After the grafting reaction, the PHB-*g*-AAc films were taken out from the glass reactors and immersed in methanol for an hour at 40°C to remove AAc monomer and ungrafted homopolymer. The degree of grafting (X_g) was calculated from the following equation[23]:

$$X_g(\%) = (W_g - W_0) \times 100/W_0$$

where W_0 and W_g are the weights of PHB films before and after graft-polymerization, respectively.

Thermal remolding

PHB-*g*-AAc films were cut into pieces (1 mm × 1 mm) and they were hot-pressed again at 190°C. The obtained film thickness was 150 μm. The thermally-remolded PHB-*g*-AAc film were isothermally crystallized at 90°C for a week before use.

Characterization of PHB-*g*-AAc

The molecular weight of irradiated PHB was measured with gel permeation chromatography (GPC) (Nihon Bunko, LC-Net II) at 38°C. Chloroform was used as the eluent and flow rate was 1.0 mL/min. Polystyrene standards with the narrow polydispersity were used to make a calibration curve. The gel fraction was calculated as follows: Gel fraction (%) = $W_g/W_0 \times 100$, where W_g is the weight of dry gel after extraction and W_0 is the initial weight of dry gel.

Mechanical properties such as tensile strength and Young's modulus of PHB films (10 mm × 50 mm) were measured at room temperature by using a Tensilon (UTM-500 from Toyo Baldwin) with a deformation rate of 40 mm/min.

Contact angle on the PHB-*g*-AAc films were measured by Contact-angle Meter (Kyowa Scientific). The contact angles were determined by the drop of 0.05*M* NaOH solution at room temperature.

The evaluation of enzymatic degradation of PHB-*g*-AAc films was carried out at 37°C in 0.1*M* phosphate buffer (pH 7.4). PHB depolymerase purified from *Ralstonia pickettii* T1[24] was used. PHB-*g*-AAc films (150 μm thick) cut into pieces of 10 mm × 10 mm were placed in small test tubes containing 1.0 mL of buffer solution (pH 7.4). The reactions were started by addition of 4 μg PHB depolymerase. The weight loss of film was measured periodically after the film was taken out and washed with methanol and distilled water.

Differential scanning calorimetry (DSC) of PHB and PHB-*g*-AAc samples was measured with Shimadzu DSC-50. About 2 mg of the sample packed in an aluminum pan was heated from 25 to 190°C under a nitrogen flow rate of 30 mL/min. The enthalpy of melting (ΔH_m) was determined from an endothermic peak at the melting point.

The surfaces of the PHB and PHB-*g*-AAc films were observed with a scanning electron microscope, SEM (Hitachi Semedx Type N), after coating of Ag with ion coater.

RESULTS AND DISCUSSION

Effect of irradiation on PHB films

Figure 1 shows the effect of irradiation dose on X_g at AAc concentration of 50 wt % after graft polymerization for 1 h. The X_g increased with the increment of irradiation dose. In previous study, enzymatic degradability of PHB powder grafted with AAc, MMA, and HEMA were investigated.[18] Enzymatic degradabilities

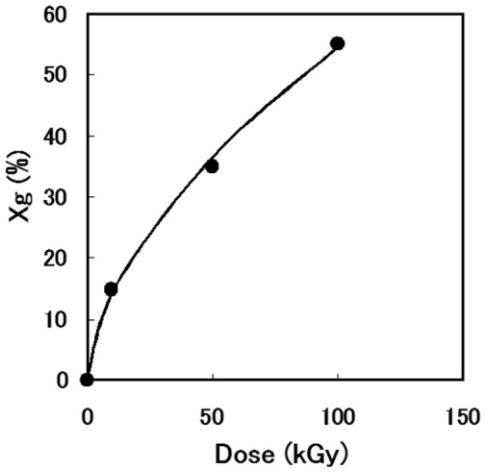

Figure 1 Relationship between irradiation dose and degree of grafting.

of these powders were affected at the X_g more than 20%. From these results, preirradiation dose of PHB films was decided to 10 kGy.

The effects of irradiation on enzymatic degradation, molecular weight, and mechanical properties were investigated. The effect of dose on enzymatic degradability of PHB films is shown in Figure 2. The enzymatic degradability of irradiated PHB film at 10 kGy was same as that of nonirradiated PHB film. The enzymatic degradability proportionally increased as the irradiation dose increased, and the enzymatic degradability after irradiation of 200 kGy became 1.5 times of intrinsic PHB.

Number-average molecular weight (M_n) and distribution of molecular weight [(M_w/M_n); M_w: weight-average molecular weight] of irradiated PHB films at various doses are listed in Table I. M_n of PHB film was steeply decreased from 3.1×10^5 to 1.4×10^5 after irradiation of 10 kGy. Then M_n decreased monotonically as irradiation dose increased. Crosslinking of PHB film didn't take place, since any gel wasn't observed after chloroform extraction.[25]

TABLE I
Molecular Weight and Mechanical Properties of Unirradiated and Irradiated PHB Films

Irradiation dose (kGy)	Molecular weight		Mechanical properties	
	M_n (10^5)	M_w/M_n	Tensile strength (MPa)	Young's modulus (GPa)
0	3.1	1.3	44.4	1.6
10	1.4	1.4	40.2	1.5
50	1.0	1.3	39.5	1.5
100	0.9	1.4	34.3	1.4
200	0.6	1.6	6.2	0.8

Tensile strengths and Young's modulus of the PHB films irradiated at various doses are also shown in Table I. There was a gradual reduction of tensile strength and Young's modulus in irradiation dose up to 100 kGy. Then, both properties dramatically decreased at irradiation dose of 200 kGy.

It was considered that promotion of enzymatic degradation was caused by decrease of molecular weight and mechanical properties by irradiation.

Grafting of PHB film with acrylic acid

To obtain the various X_g, the grafting was carried out at different concentration of AAc at 10 kGy. Figure 3 shows the effect of AAc concentrations on X_g for 3 and 5 h grafting reaction. After 3 h reaction, X_g of 2% at 10 wt % AAc increased to 15% by the increasing the AAc concentration up to 50 wt %. There were linear relationship between X_g and AAc concentration.

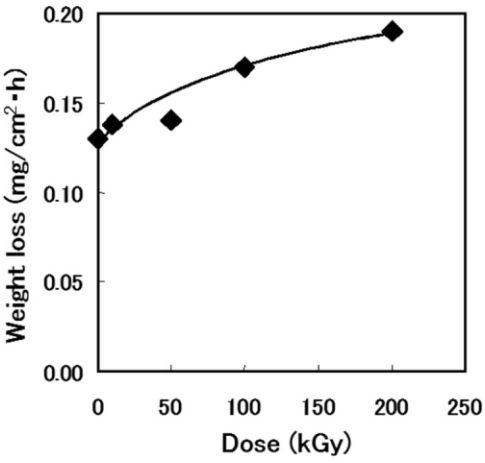

Figure 2 Enzymatic degradation of PHB films irradiated at various doses.

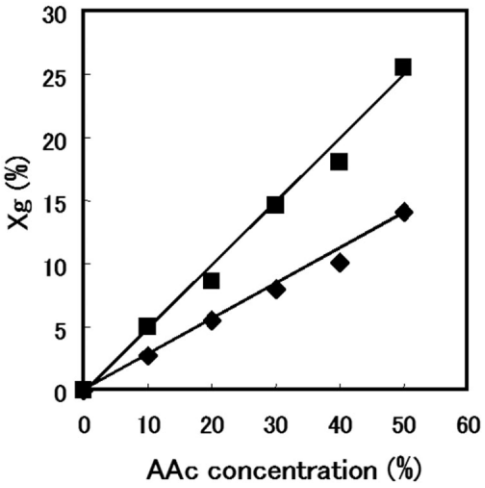

Figure 3 Relationship between AAc concentration and degree of grafting; (♦): after 3h, (■): after 5h.

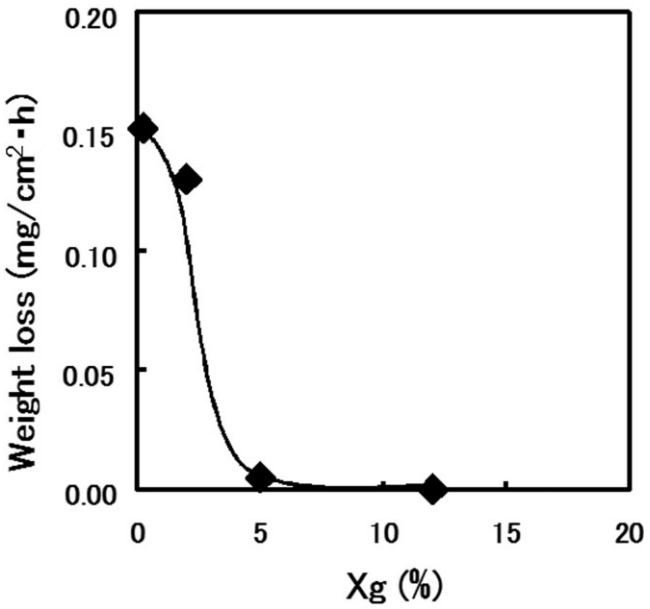

Figure 4 Enzymatic degradation of PHB-*g*-AAc films.

In this study, grafted PHB films having X_g of 2, 5, and 15% were obtained at AAc concentration of 5, 10, and 30 wt % for 3 h grafting reaction, respectively. X_g of 25% was obtained at AAc monomer concentration of 50 wt % for 5 h grafting reaction.

Enzymatic degradation of PHB-*g*-AAc

The resulting PHB-*g*-AAc films were degraded by enzyme. Figure 4 shows the relation between X_g and weight loss in the enzymatic degradation of PHB-*g*-AAc film. The degradation rate of a intrinsic PHB film was 0.15 mg/cm^2 h. The result showed that weight loss of PHB-*g*-AAc films was considerably reduced with increase of X_g. The biodegradability of PHB-*g*-AAc was almost lost as degree of grafting higher than 5%.

To investigate the effect of remolding on PHB-*g*-AAc films, the PHB-*g*-AAc films were remolded by a hot-press again. Figure 5 shows the plot of weight loss of remolded PHB-*g*-AAc against X_g of PHB-*g*-AAc. The enzymatic degradability increased as the X_g increased up to 12%. The weight loss of intrinsic PHB and PHB-*g*-AAc films with X_g of 12% were 0.16 and 0.21 mg/cm^2 h, respectively. However, the enzymatic degradability decreased with further increasing of X_g. In a previous study, Doi and coworkers found that the number-average degree of polymerization (P_n) of PHB decreased during isothermal degradation around the melting point.[26] When we compare with the enzymatic degradability of thermally-remolded PHB-*g*-AAc films, it has possibility to affect of thermally degradation. Therefore, intrinsic PHB film also remolded and investigated the enzymatic degradation.

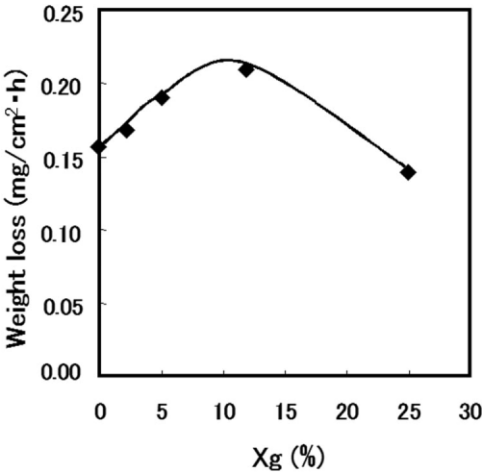

Figure 5 Enzymatic degradation of thermally remolded PHB-*g*-AAc films.

As a consequence, the enzymatic degradability PHB-*g*-AAc films was recovered by remolding. The recovery of enzymatic degradation clearly observed at the grafted samples with X_g ranging from 5 to 10%. In this case, the graft chains disturbed biodegradation of PHB-*g*-AAc is dispersed in remolded PHB.

SEM observation

Figure 6 shows the surfaces of PHB film, PHB-*g*-AAc film, and thermally-remolded PHB-*g*-AAc film after enzymatic degradation for 24 h. It could be seen that,

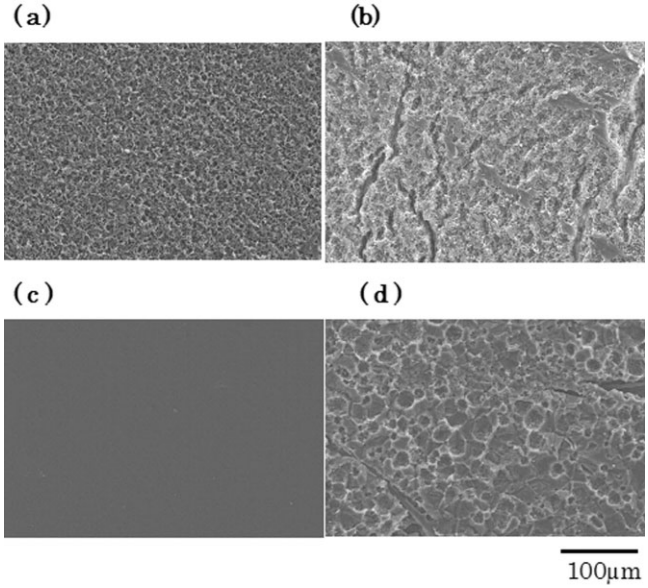

Figure 6 SEM micrographs of the surface of PHB and PHB grafted with AAc films after enzymatic degradation for 24 h. (a) PHB film; (b) X_g = 2% PHB-*g*-AAc film; (c) X_g = 5% PHB-*g*-AAc film; (d) X_g = 5% thermally remolded PHB-*g*-AAc film.

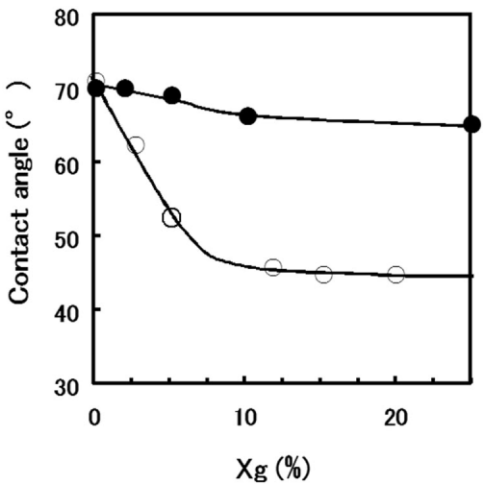

Figure 7 Contact angle of PHB films grafted with AAc; (○): PHB-*g*-AAc films, (●): thermally remolded PHB-*g*-AAc films.

a PHB film degraded and outline of spherulites appeared clearly [Fig. 6(a)], and PHB-*g*-AAc film of 2% X_g also degraded and appeared spherulites, whose size is similar to that of PHB film [Fig. 6(b)]. The surface of grafted PHB with X_g of 5% was smooth [Fig. 6(c)]. It indicated that this film didn't degrade by PHB depolymerase. However, the remolded PHB-*g*-AAc with X_g of 5% degraded more than PHB [Fig. 6(d)]. Spherulite size of remolded PHB-*g*-AAc with X_g of 5% was larger than that of PHB film. It was confirmed that the enzymatic degradability of remolded PHB-*g*-AAc was recovered.

Contact angle of the film

Figure 7 shows the contact angles of PHB-*g*-AAc films, before and after thermal remolding. Contact angle of PHB-*g*-AAc films was considerably decreased by increasing of X_g. At more than 12% of X_g, contact angle became constant. It might caused by the surface of PHB film was completely covered with grafted acrylic acid. However, the contact angle of remolded PHB-*g*-AAc films decreased slightly.

As described above, the enzymatic degradability of PHB-*g*-AAc films was considerably reduced with increase of degree of grafting and it was lost at X_g of 5%. Contact angle of PHB-*g*-AAc films was steeply decreased up to X_g of 5%. This result implies that grafted hydrophilic AAc cover the almost the whole surface of PHB film. Hence, the enzymatic degradation was retarded by grafting. On the other hand, the enzymatic degradability of remolded PHB-*g*-AAc films was recovered, and the contact angle of remolded PHB-*g*-AAc films was slightly decreased. This means that the grafting chains were homogenously distributed in the remolded film. In the surface of remolded PHB-*g*-AAc films, PHB appeared and was easily degraded by enzyme.

Thermal analysis of PHB-*g*-AAc

Table II lists X_g, melting points (T_m), and enthalpies of melting (ΔH_m) of PHB-*g*-AAc, before and after thermally-remolded treatment. PHB is a crystalline polymer, on the other hand, grafting chains, PAAc, is a noncrystalline polymer. The ΔH_m may be corrected by weight fraction of PHB in the grafted samples according to the following equation[18,19]:

$$\text{Corrected enthalpy of melting}(\Delta H_m)_{\text{corr}} = \Delta H_m/(1-X_g/100)$$

T_m and $(\Delta H_m)_{\text{corr}}$ values of PHB-*g*-AAc films hardly changed with increase of X_g. The results showed that graft chains had little effect on the crystalline structure.

To compare with intrinsic PHB films before and after thermal remolding, it was confirmed that T_m decreased and ΔH_m increased after thermal remolding. Lowering of T_m was caused by decrement of molecular weight for the thermal degradation. As a result, PHB film was crystallized easily and ΔH_m increased after thermal remolding.

$(\Delta H_m)_{\text{corr}}$ value of thermally-remolded PHB-*g*-AAc films decreased as X_g increased. This result indicates that graft chains restricted a crystallization of PHB

TABLE II
Thermal Properties of PHB Films Grafted with AAc

Sample no.	X_g	PHB-*g*-AAc film			Thermally-remolded PHB-*g*-AAc film		
		T_m (°C)	ΔH_m (J g^{-1})	$(\Delta H_m)_{\text{corr}}$ (J g^{-1})	T_m (°C)	ΔH_m (J g^{-1})	$(\Delta H_m)_{\text{corr}}$ (J g^{-1})
1	0	174.0	70.7	70.7	170.8	73.4	73.4
2	2	170.5	69.9	71.3	169.9	67.3	68.7
3	5	170.6	66.1	69.6	168.6, 173.7	60.7	63.9
4	12	170.1	60.3	68.5	168.5, 173.7	51.9	59.0
5	25	170.8	53.6	69.2	167.7, 173.1	46.9	60.5

molecule during the remolding. T_m of remolded PHB-*g*-AAc films indicated the double peaks at X_g more than 5%. It implies the formation of two crystal modifications. The higher temperature peak was formed by original crystal modification, and the lower temperature peak was contributed to the more disordered crystal regions, which were induced by grafted polymer chains.

On the point of view of crystallinity, graft chains covered with the surface of PHB film had little effect on crystallinity of PHB film. Amorphous region of PHB-*g*-AAc films increased after the thermally-remolded treatment, since graft chains disturbed the crystallization of PHB.

CONCLUSIONS

Enzymatic degradability of PHB was controlled by grafting of AAc and thermal remolding. Nondegradable AAc grafting chains of X_g more than 5% covered the surface of PHB film and retarded the biodegradability from PHB films. In this film, PHB depolymerase couldn't contact the surface of PHB. In consequence, the enzymatic degradability of PHB-*g*-AAc film was disappeared. Thermally-remolded PHB-*g*-AAc film recovered the enzymatic degradability. After the thermal remolding, graft chains in the surface were incorporated at the amorphous region in the film and new PHB surface revealed.

For the thermal analysis, it was confirmed that $(\Delta H_m)_{corr}$ decreased with increment of X_g after thermal remolding. This decrease of crystallinity was caused by incorporation of AAc grafting chains at the amorphous region in PHB film. As a result, the enzymatic degradability of remolded PHB-*g*-AAc film is higher than intrinsic PHB film in the case of X_g from 5 to 10%.

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