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Thermal Properties and Biodegradability Studies of Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)

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Abstract For investigating the relationship between thermal properties and biodegradability of poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), several films of PHBV containing different polyhydroxyvalerate (HV) fractions were subjected to degradation in different conditions for up to 49 days. Differential scanning calorimetry (DSC), thermogravimetry (TG), specimen weight loss and scanning electron microscopy (SEM) were performed to characterize the thermal properties and enzymatic biodegradability of PHBV. The experimental results suggest that the degradation rates of PHBV films increase with decreasing crystallinity; the degradability of PHBV occurring from the surface is very significant under enzymatic hydrolysis; the crystallinity of PHBV decreased with the increase of HV fraction in PHBV; and no decrease in molecular weight was observed in the partially-degraded polymer.

Keywords Poly(β -hydroxybutyrate-co-hydroxyvalerate) · Biodegradable material · Thermal properties · Enzymatic degradation

Introduction

Plastics have been widely utilized in almost every manufacturing industry. It is estimated that nearly 150 million tons of plastics are produced very year [1], and several million tons of plastics are discarded into the biosphere. This poses a threat to biotic and abiotic components of the environment [2]. In spite of the increasing efforts to decrease accumulation of wastes and recycle them, more and more damage is done on the environment. As an alternative to chemical-based, non-biodegradable plastics, more and more attentions are paid to the environmentally-friendly biodegradable plastics, which can help overcome some pollution problems and reduce petroleum dependency.

Polyhydroxyalkanoates (PHA_S), the polymers synthesized by microorganisms using renewable resources as carbon feedstocks, are currently widely recognized among biodegradable polymers, which have numerous useful properties and a wide range of application [3–5]. Polyhydroxybutyrate (PHB), one type of PHA polymer, is probably the most extensively studied biodegradable thermoplastic polymer. However, practical application of PHB has often been limited by its narrow processing window and brittleness [6–8]. Careful control of the fermentation conditions and the feeding of carbon source used has led to the synthesis of a variety of PHA copolymers, including poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV), with improved physical properties. PHBV is commercially available and has physical and mechanical properties that are comparable to the conventional thermoplastics such as

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poly(L-lactic acid) (PLA) [9] and poly(butylene succinate) [10]. Before their applications can be widespread, comprehensive studies on the biodegradability and degradation mechanisms of these polymers are necessary. Up to now, there has been lots of research on the degradation of PHAs or PHA blended with other materials in a marine environment [11, 12], tropical mangrove ecosystem [13], soil environment [14–17], enzyme solution [18–20], and microbial environment [21–23]. Different degradation behaviors of PHAs were found in different degradation processes, even for a same polymer. It has been reported that the molecular weight of polyhydroxybutyrate (PHB) chains decreased during marine degradation [12] and enzymatic degradation [18]. However, its molecular weight was seen not to decrease in another enzymatic degradation study [24]. This may result from the differences in the environment and mechanism of degradation.

There are several factors that contribute to the enzymatic degradation rate of PHBV. In general, the biodegradability of PHAs is influenced not only by the chemical structure, but also by the physical and thermal properties. The chemical structure, surface condition of the polymer, and their related physical properties such as: crystallinity, crystal structures, molecular orientation, melting temperature (T_m), and glass transition temperature (T_g) are known to have crucial effects on the PHAs biodegradation of polyesters. Hence, much attention should be given to investigate the relationship between crystallinity and biodegradation rate of PHA copolyesters. However, very few investigations of the thermal properties and biodegradability of PHBV of various HV fractions have been reported to date.

The aim of this study was to investigate the relationship between the biodegradation behavior and thermal properties of PHBV specimens. The thermal properties of PHBV specimens were measured by various characterization techniques, such as differential scanning calorimetry (DSC) and thermogravimetry (TG). The biodegradability of PHBV films was investigated during incubation in different media, and the surface topography of films was analyzed at different times during degradation using scanning electron microscopy (SEM). Weight loss and average molecular weight were also monitored to characterize the extent of degradation.

Experimental Section

Materials and PHBV Films

PHBV (containing 3-hydroxyvalerate monomer (HV) fractions of 4.6, 9.5, and 20.7%, respectively) polymers were produced by cultures of *Ralstonia eutropha* fed with

glucose and valerate as carbon sources. PHBV films were prepared by solvent casting in chloroform. The solution of PHBV (0.02 g/ml) was cast on petri plates, and the solvent was allowed to evaporate in a controlled air stream for 12 h. Polymer was kept at room temperature for >2 weeks to reach equilibrium of crystallinity. The final thickness of PHBV film specimens were approximately 0.2 mm. Specimens of 2 cm² dimensions were cut from the PHBV films.

Analysis Methods

Thermal properties of PHBV were analyzed by DSC (TA instruments, Model 2910) and TG (Model Q50). Specimens weighing approximately 3 mg were used for the DSC study. Heating and cooling rates were maintained at 10 °C/min during the DSC runs. Specimens were heated from –30 to 200 °C for 3 min. Melting temperature (T_m), melting enthalpy (ΔH_m), crystallization temperature (T_c), crystallization enthalpy (ΔH_c), and glass transition temperature (T_g) were obtained from the thermograms. Specimens weighing approximately 4 mg were used for the thermogravimetry (TG) study. Specimens were heated from 0 to 400 °C at a rate of 10 °C/min during the TG runs in order to test the temperature of thermal degradation.

In the lipase degradation analysis, PHBV specimens of similar shape and specific area were placed into pure PBS (phosphate buffered saline, pH = 7.3) and PBS containing 1 g/L lipase (LEVEKING, Shenzhen, China, enzyme activity: 10 KLU/g) at 37 °C. Degradation studies were carried out on 7-well plates with same volume per well, which contained three PHBV specimens, each with different HV content. The PBS containing lipase was changed every 2 days to avoid changes in lipase concentration due to possible evaporation of water during degradation. Weight loss measurements were performed every 7 days. For this procedure, specimens were removed, washed with distilled water 3 times, followed by drying in vacuo to a constant weight before analysis.

The dried specimens were also used for other subsequent analyses. Specimens were dissolved in chloroform, and the intrinsic viscosity of the solution was measured at 30 °C using an Ubbelohde type capillary viscometer. Weight average molecular weight was calculated according to the Mark-Houwink equation [25].

The SEM of PHBV was obtained following gold coating by the use of a scanning electron microscope (Hitachi S-570). The surface topography and the porosity of the membranes were examined using the micrographs.

All data were expressed as means \pm standard deviation (SD) for $n = 5$ and were verified using standard analysis of Student's t test.

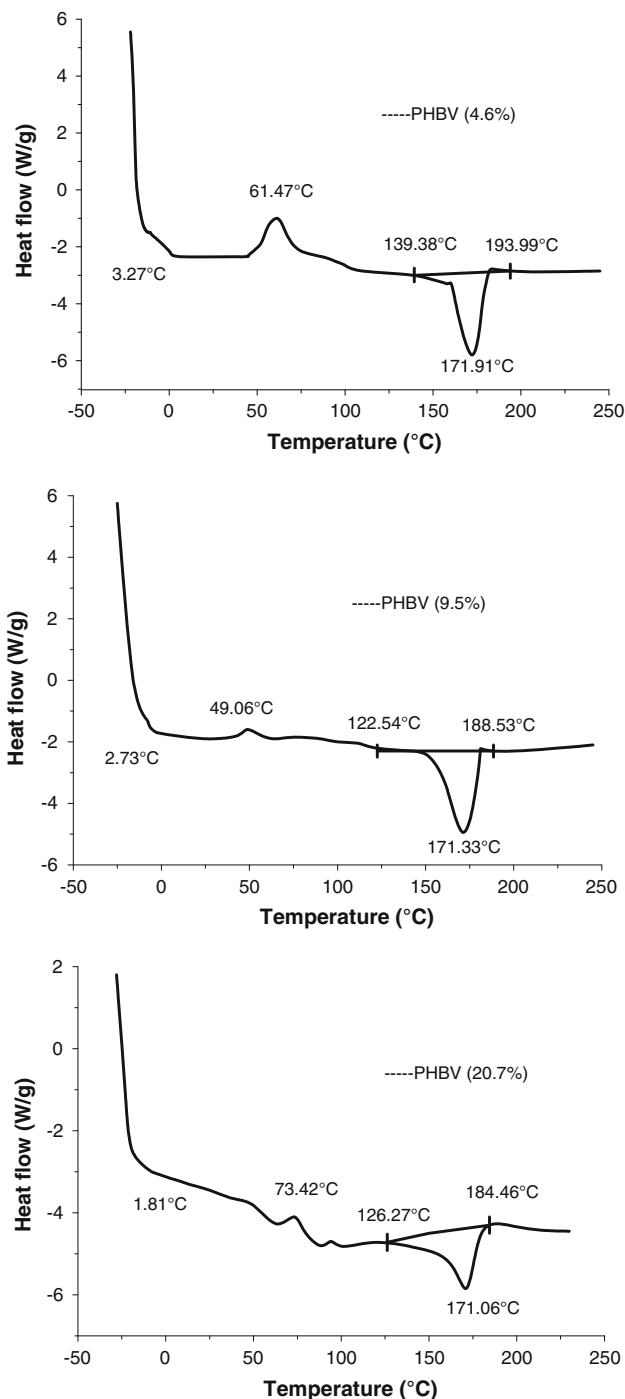


Fig. 1 DSC thermograms of different PHBV specimens

Table 1 Thermal properties of different PHBV specimens

Sample	T _g (°C)	T _c (°C)	T _m (°C)	ΔH _c (J/g)	ΔH _m (J/g)
PHBV (4.6 mol% HV)	3.27 ± 0.81	61.47 ± 1.43	171.91 ± 1.44	23.20 ± 0.22	34.43 ± 1.14
PHBV (9.5 mol% HV)	2.73 ± 0.52	49.06 ± 1.78	171.33 ± 1.77	16.49 ± 0.41	65.83 ± 0.88
PHBV (20.7 mol% HV)	1.81 ± 0.64	73.41 ± 1.02	171.06 ± 1.13	7.13 ± 0.30	28.28 ± 1.37

Results and Discussion

Thermal Properties of PHBV

The thermal properties of PHBV (containing 4.6, 9.5 and 20.7 mol% of HV monomer, respectively) were analyzed by the DSC and TG. Figure 1 shows the DSC thermograms of the different PHBV specimens. The vitrification point, melting temperature, melting enthalpy, crystallization temperature, and crystallization enthalpy of PHBV specimens were summarized in Table 1. It can be seen that these three PHBV specimens have no obvious differences in T_g and T_m. However, their T_c and ΔH_c are very different from each other. That is to say, the three polymers exhibited obvious differences in crystallinity. The data in Table 1 suggests that the crystallinity of PHBV became higher with decreasing HV content in the polymer, and the crystalline peak became narrower with increasing HV content (Fig. 1). The melting point of PHBV can also become lower with an increase of HV fraction. This results in an improvement in the ductility and flexibility of the polymer. However, in the three samples discussed in Fig. 1 and Table 1, a change in melting temperature is not observed.

The results of TG graph analysis are listed in Table 2. It can be seen from Table 2 that the thermal decomposition temperature of three PHBV specimens were in the range of 240–290 °C, and decreased with increasing HV content. The three PHBV specimens did not start thermal decomposition until 220 °C, which is similar to the previous report [26]. The thermal weight loss of three PHBV specimens was higher than 90%. The final degradation products were CO₂ and H₂O.

Because the melting temperature of three PHBV specimens were around 170–180 °C and their thermal decomposition temperature were above 220 °C, the processing window becomes very wide, compared to PHB with a narrow processing window [15]. This again suggests that the workability could be greatly improved with increasing HV content in PHBV polymer.

Enzymatic Degradation of PHBV

It is known from studies of biocompatibility of PHA in mammalian tissues that non-specific lipases can aid in

Table 2 Summary of TG graph analysis

Sample	Teoi (°C)	Tem (°C)	Teof (°C)	Wt-loss (%)
PHBV (4.6 mol% HV)	263.52 ± 1.14	288.71 ± 1.53	302.86 ± 1.89	95.68 ± 1.84
PHBV (9.5 mol% HV)	247.58 ± 1.77	273.42 ± 1.19	288.79 ± 1.36	98.43 ± 0.91
PHBV (20.7 mol% HV)	236.16 ± 1.71	240.10 ± 1.03	249.40 ± 1.61	91.97 ± 1.10

Teoi initial thermal decomposition temperature, *Tem* onset temperature, *Teof* finished thermal decomposition temperature, *Wt-loss* thermal weight loss

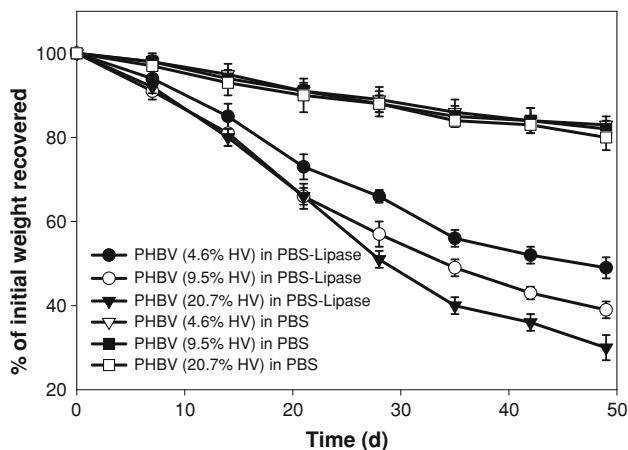


Fig. 2 Weight loss profile of PHBV film in PBS and PBS-Lipase medium

degrading PHA polymer. Thus, we attempted biodegradability studies using lipase. In the degradation studies, the biodegradability of polymer was investigated by inserting PHBV films into PBS medium or PBS-Lipase medium. Fig. 2 shows the PHBV specimen weight as a function of degradation time and provides direct evidence of PHBV degradation in the two different media. It can be seen that the PHBV specimens only lost about 18% of their original weight in the pure PBS medium after 7 weeks. However, a weight loss of about 50, 61, and 70% was observed in PBS-Lipase medium for the PHBV containing an HV fraction of 4.6, 9.5, and 20.7 mol%, respectively. The degradation became so severe that the PHBV specimens broke into pieces and could not be recovered from the PBS-Lipase medium when the degradation study was extended to beyond 50 days. It is confirmed that the biodegradation rate of PHBV, as indicated by the slope of the curves in Fig. 2, increases with increasing HV content and degradation time.

In general, two types of mechanisms are possible for polymers that are exposed to a medium that facilitates degradation: hydrolytic and enzymatic. Both mechanisms involve polymer chain cleavage and result in molecular weight reduction. In order to investigate the degradation mechanism, the molecular weights of partly degraded PHBV specimens were measured as discussed above. No decreases in molecular weight were observed in this study (data not shown). This was attributed to the fact that

enzymes cannot permeate the macromolecular lattice system of polymers, and enzymatic degradation only occurred at the surface of polymers where erosion or weight loss can occur. Similar results were found in previous studies [20, 24]. Enzyme molecules were able to diffuse and degraded polymer first at the amorphous regions and finally at the crystalline regions. Because degradation only occurred on the polymer surface and degradation products were removed from the surface by watery medium surrounding the polymers, the molecular weights exhibited no change.

In Fig. 3, we present the SEM photomicrographs showing the surface topography of the specimen of PHBV with 20 mol%HV before, different periods during and after degradation. It can be seen from Fig. 3a that the surface of film was relatively smooth except for a few scratches before degradation. However, after degradation in the PBS-Lipase medium the surface of the specimens, as shown in Fig. 3b, became rougher with corrosion and weight loss on the specimen surface. The number of pits dramatically increased and in size and depth, as the degradation process continued. The degradation occurred not only on the specimen surface but also diffused into the pits and degraded the specimen totally. It can be seen that more lipase and water molecules were able to fill the larger pits, leading to further degradation (Fig. 3c). These results are similar to those found in the studies conducted in previous works [27, 28]. It has been reported that the rate of degradation of PHAs was dependent on the surface area of the polymer exposed to enzymatic hydrolysis. Enzymatic hydrolysis of polymer began on the surface and at physical lesions and proceeded to the inner part of the material [27]. Similarly, the biodegradation of PHB films proceeded via surface erosion mechanisms and PHB films were biodegraded homogeneously on the surface where the marine microbes attached, resulting in the formation of pits [28].

Conclusions

The thermal properties and biodegradability of PHBV polymers with different monomer contents were characterized using various techniques and methods including DSC, TG, SEM, weight loss and molecular weight measurements. The HV fraction in PHBV has a

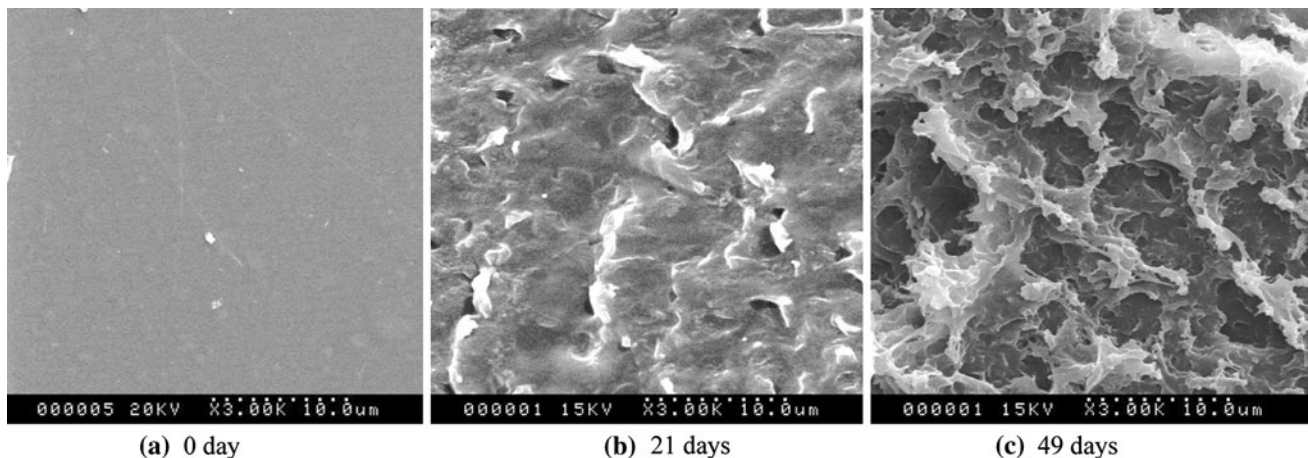


Fig. 3 SEM photomicrographs of the surface of 20.7 mol%PHBV films at different degradation time

profound influence on the thermal properties of the polymer. When the HV fraction in PHBV increased, the crystallinity of the polymer decreased. As a result, both the degradation rate and the workability of the PHBV polymer were improved. The loss of specimen weight and roughening of the surface topology as shown by the SEM photomicrographs has confirmed that PHBV was degraded mostly at the surface, including the damaged areas, in PBS-Lipase medium.

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