

Material Behaviour

Biodegradation behavior of PHAs with different chemical structures under controlled composting conditions

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ABSTRACT

Polyhydroxyalkanoates (PHAs) are biobased and biodegradable polymers, of which poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV, 3 %mol HV) and poly-3-hydroxybutyrate-co-4-hydroxybutyrate [P(3HB,4HB), 10 %mol 4HB] are currently commercialized. To understand the influence of chemical structure on the biodegradability of PHA, the biodegradation behavior of poly(hydroxybutyrate) (PHB), PHBV (40 %mol HV), PHBV (20 %mol HV), PHBV (3 %mol HV) and P(3HB,4HB) (10 %mol 4HB) were investigated under the controlled composting conditions according to ISO 14855-1. It was found that PHAs with different chemical structures can be biodegraded under the controlled composting conditions. The order of biodegradability was P(3HB, 4HB) (40 %mol 4HB) \approx PHBV(40 %mol HV) > PHBV(20 %mol HV) > PHBV(3 %mol HV) > PHB.

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1. Introduction

Poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV) is one type of polyhydroxyalkanoate (PHA) that can be produced by bacteria from agricultural raw materials. The chemical structures of several PHAs are shown in Fig. 1. The biobased PHBV can be processed to form a variety of useful products and has attracted the attention of academia and industry due to its good biodegradability, biocompatibility and thermo-plasticity. Compared with poly(hydroxybutyrate) (PHB), PHBV has good toughness and ease of processing. However, its poor thermal stability, low elongation at break, slow crystallization rate and high degree of crystallinity still remain problematic. Therefore, it has been subject to chemical and physical modifications.

In the chemical modifications, the HV content of PHBV can be adjusted by controlling the amount of propanoic acid during the biosynthesis process [1–3]. Other chemical methods, such as grafting and reactive blending have also

been used to modify its properties [4–10]. It was found that the melting temperature of PHBV decreased with increase of HV content when the total molar content of HV was less than 28%, but increased when the molar content of HV was more than 28% [11]. The toughness of PHBV was also affected by the HV content in the molecular chains. It could vary from rigid to flexible with change of HV content, for example, it could behave like polyethylene, polypropylene and polyvinylchloride when the HV content is high, medium and low, respectively [12].

Obviously, the modification of PHBV should not destroy its biodegradability, which is its most important advantage, regardless of the aims of modification. Therefore, broad investigation of biodegradability of different modified PHBV systems is important.

Hermida et al. [13] found that the biodegradation of PHBV proceeded via a surface erosion mechanism resulting in the formation of pits by microbial attack, and the crystallinity and molecular weight of PHBV specimens remained virtually constant after 45 days of degradation in a liquid medium inoculated with a 2% v/v fresh spore suspension of *Somaiyaensis* SSM 5670. Li et al. [14] studied the enzymatic

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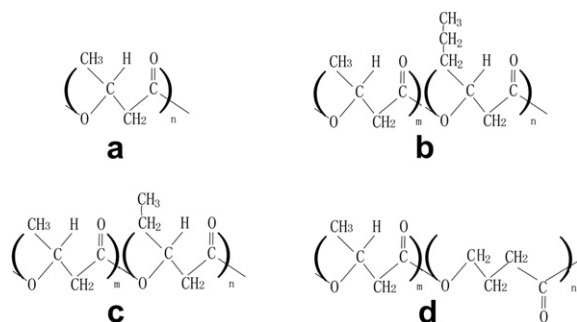


Fig. 1. The chemical structures of PHA: (a) P(3HB); (b) P(3HB, 3HH); (c) P(3HB, 3HV); (d) P(3HB, 4HB).

Table 1

The characteristics of PHA's used in this paper.

Sample	Size μm	Molecular weight (Mn) $\times 10^4$ g/mol	Tg $^{\circ}\text{C}$	Tm $^{\circ}\text{C}$	Density g/cm^3
PHBV-3 (3 %mol HV)	1–80	40.4	6	160	1.24
PHBV-20 (20 %mol HV)	1–80	32.4	–2	130	1.24
PHBV-40 (40 %mol HV)	1–80	32.4	–12	90	1.24
PHB	1–90	24.0	–	175	1.23
P(3HB, 4HB)	Powder	44.6	3	164	1.23

degradation of microbial copolyesters consisting of 3-hydroxybutyrate and medium-chain-length 3-hydroxyalkanoates. They found that the crystallinity of PHAs decreased in the order of PHB > PHBV (19 %mol HV) > PHBHHx (19 %mol HHx) > PHBA (15 %mol HA), while the degradation rate increased in the order of PHBV > PHB > PHBHHx > PHBA in a medium with PHA depolymerase. They proposed that the length of side chains in the PHA structure would affect the depolymerase degradation rate, that is, PHA with longer side chains, including PHBHHx and PHBA, are less favorable for depolymerase degradation than PHA with shorter side chains. Weng et al. investigated the biodegradation of PHBV (3 %mol HV) on both pilot and laboratory scale composting conditions and found that its degradation occurred from the surface erosion to the interior [15].

Although there have been some investigations on the biodegradation of PHA, the effect of HV content on the biodegradation of PHA has not been reported in open literature. Therefore, in this paper, the biodegradability of PHA with different chemical structures PHBV (40 %mol HV), PHBV (20 %mol HV), PHBV (3 %mol HV), P(3HB, 4HB) has been investigated by the controlled composting conditions according to ISO 14855-1 [16].

2. Experimental

2.1. Materials

All PHA's used in this study were supplied by Ningbo Tianan Biomaterials Co. Ltd. China and their properties are listed in Table 1.

2.2. Preparation of PHA film

3 g PHA powder was dissolved in 60 mL chloroform at 60 $^{\circ}\text{C}$ for 3 h. The solution was then poured evenly onto a glass plate in a fume hood. After 3 h, a thin film was recovered from the glass plate. The film was then dried in an oven at 60 $^{\circ}\text{C}$ for 24 h in preparation for testing.

2.3. Biodegradation under controlled composting conditions

The biodegradation testing of PHA was carried out according to the method described in ISO 14855-1:2005, which is a procedure to determine the ultimate biodegradability of material under conditions simulating an intensive aerobic composting process. The scheme of biodegradation testing apparatus is shown in Fig. 2. It can be seen that the test apparatus contains composting vessels (2.5 L), an air-supply system and the apparatus for determination of carbon dioxide with a continuous infrared analyzer. 100 g of test material was mixed with 600 g of inoculum and then introduced into a static composting vessel where it was intensively composted under the optimum oxygen level using an air-supply system. The concentration of oxygen should not be lower than 6%, and the temperature was set at 58 $^{\circ}\text{C} \pm 2$ $^{\circ}\text{C}$. The test period should not exceed 6 months. The compressed air was supplied at a constant low pressure at a rate of 150 mL/min.

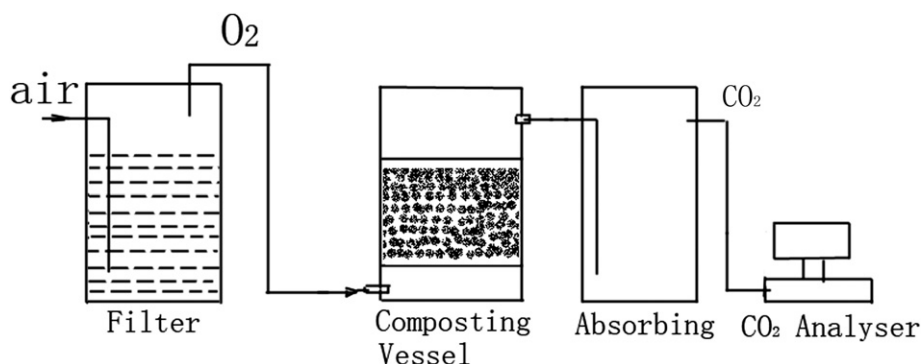


Fig. 2. The apparatus under the controlled composting testing.

Table 2
The basic characteristics of compost used as inoculums.

Inoculums (g)	Dry solids (g)	Volatile solids (g)	pH	Ratio of carbon to nitrogen (C/N) (g)
600	314.10	86.88	8.2	14.1

The carbon dioxide in the air was first removed by passing the air through a solution of sodium hydroxide, and the air was humidified at the same time.

Two month old mature compost which was taken from the municipal organic waste composting factory in Beijing and sieved with a screen of 0.5 cm–1 cm was used as inoculum. The basic properties of the mature compost are listed in Table 2. It can be seen that the percentage of total

dry solids of inoculum was 52.4% and the percentage of volatile solids was 14.5%. The pH value of the inoculum was 8.2. All the properties of the compost matched with ISO 14855-1 requirements.

The carbon dioxide produced from biodegradation of the organic materials in the composting vessel was measured at regular intervals to determine the cumulative carbon dioxide production. The percentage of biodegradation of a test sample was determined as the ratio of carbon dioxide detected from biodegradation of the material to the maximum theoretical amount of carbon dioxide that could be released by the test material. The theoretical amount of carbon dioxide produced during biodegradation of the sample was calculated from the measured total organic carbon (TOC) content determined by an elemental analyzer (MT-5CHN, Japan Yanaco Co.).

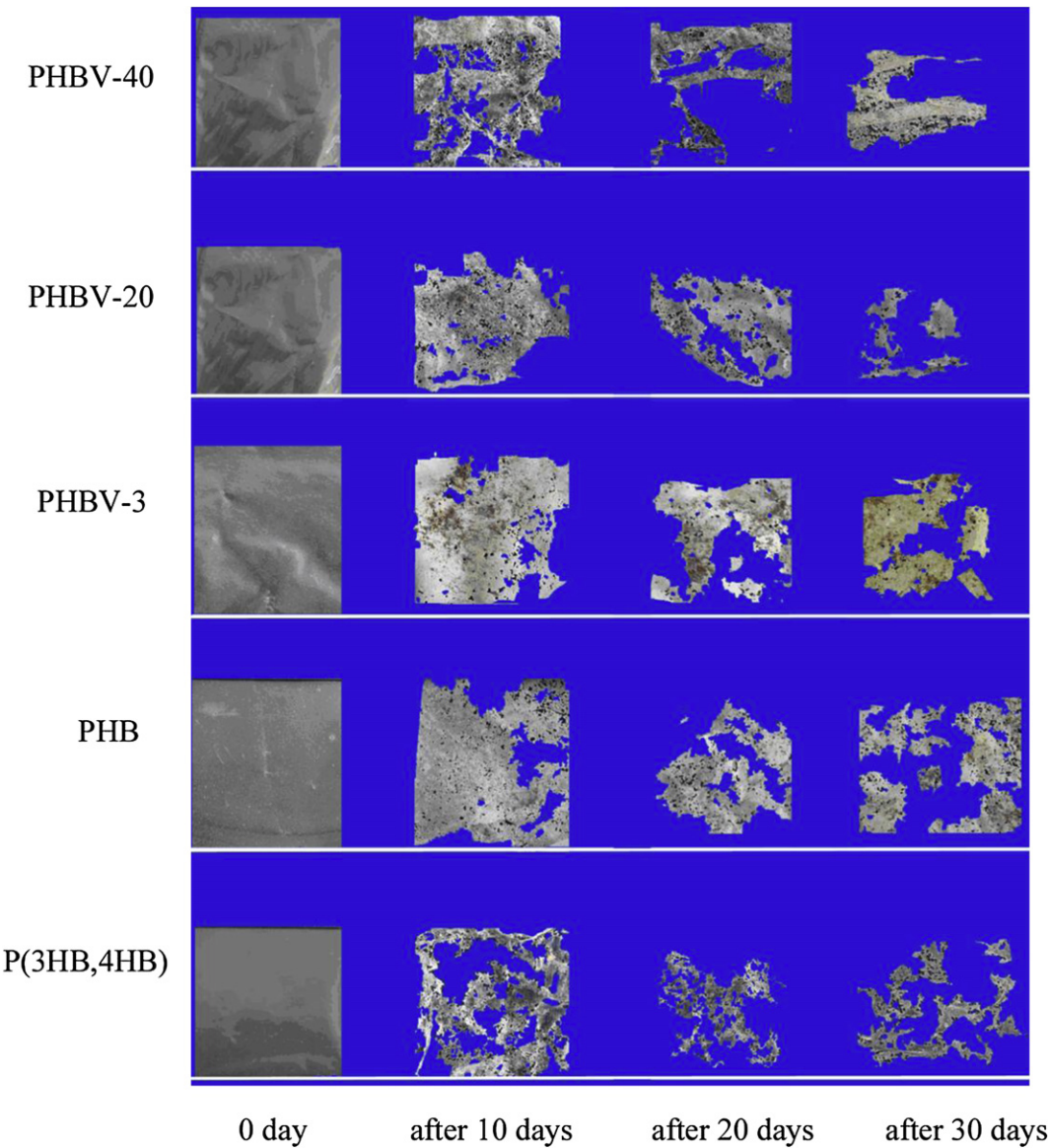


Fig. 3. The apparent variation of PHA films in the controlled composting condition.

The theoretical amount of carbon dioxide ($ThCO_2$) produced by the test material, was calculated using Equation (1):

$$ThCO_2 = M_{TOT} \times C_{TOT} \times \frac{44}{12} \quad (1)$$

where M_{TOT} is the initial total dry solids in the test material, in grams, C_{TOT} is the proportion of total organic carbon in the total dry solids in the test material, in grams per gram; 44 and 12 were the molecular mass of carbon dioxide and the atomic mass of carbon, respectively.

The biodegradation percentage (D_t) of the test material for each measurement interval determined from the released cumulative amounts of carbon dioxide was calculated according to Equation (2):

$$D_t = \frac{(CO_2)_T - (CO_2)_B}{ThCO_2} \times 100 \quad (2)$$

where $(CO_2)_T$ was the cumulative amount of carbon dioxide evolved in each composting vessel; $(CO_2)_B$ was the mean cumulative amount of carbon dioxide evolved in the blank

vessels; $ThCO_2$ was the theoretical amount of carbon dioxide produced by the test material.

The biodegradation percentage did not include the amount of carbon converted to new cell biomass which was not turned into carbon dioxide through metabolization during the test.

2.4. Characterization

The surface morphology of biodegraded samples at different composting times was investigated with a digital camera (Sony DSC-W80, Japan) and scanning electron microscope (TESCAN VEGA II, TESCAN, s.r.o., Czech). FT-IR spectra of biodegraded samples were obtained with an Avatar 370 FT-IR spectrophotometer (Nicolet Co., USA) using a potassium bromide pellet. The thermal decomposition temperatures of biodegraded samples were determined with a TA Universal V4.5A TGA (TA, USA) at a heating rate of $10^\circ\text{C min}^{-1}$ from 40 to 600°C under a nitrogen atmosphere. The molecular weights of PHA were determined by a GPC

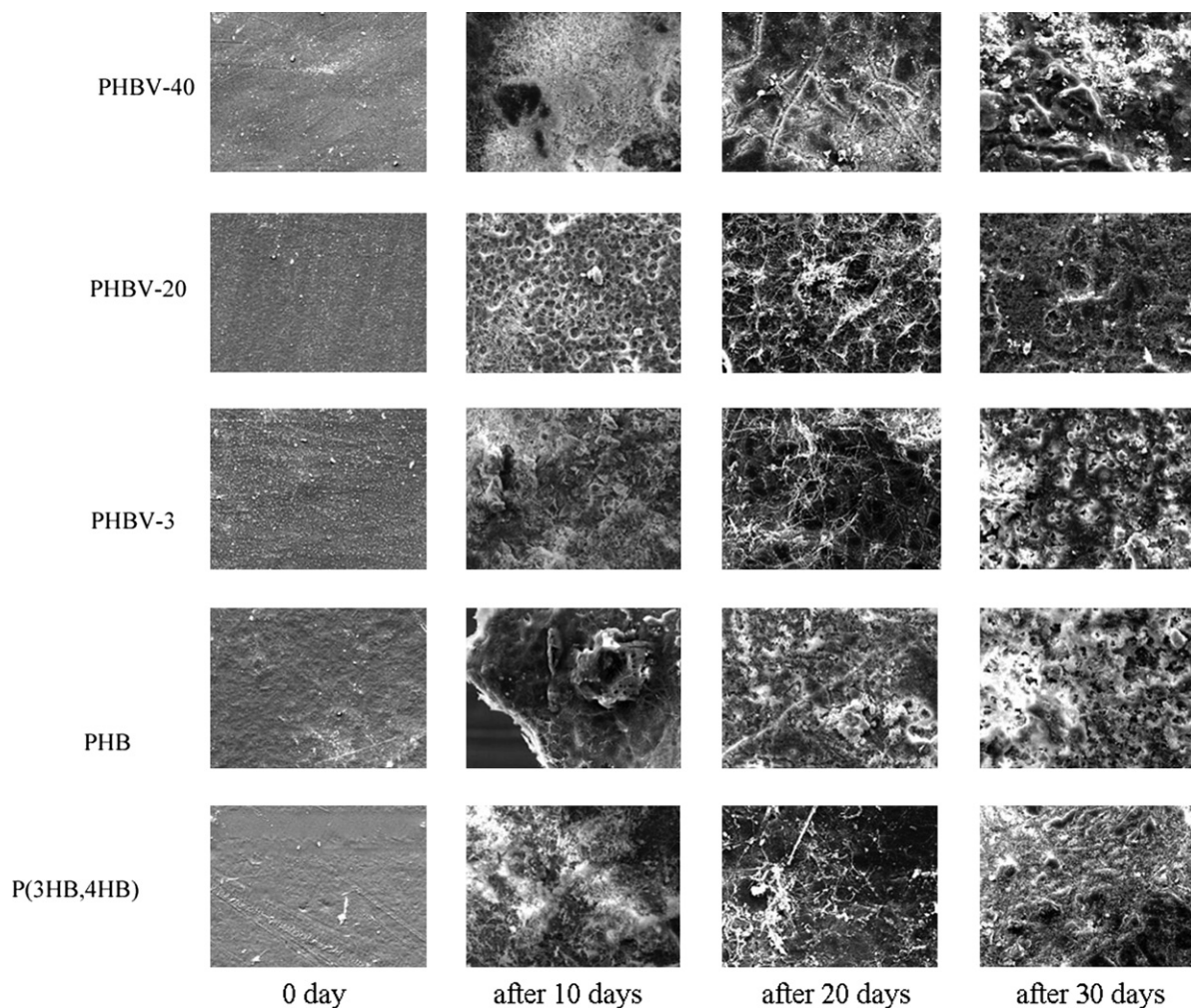


Fig. 4. SEM pictures of PHA samples at different degradation time.

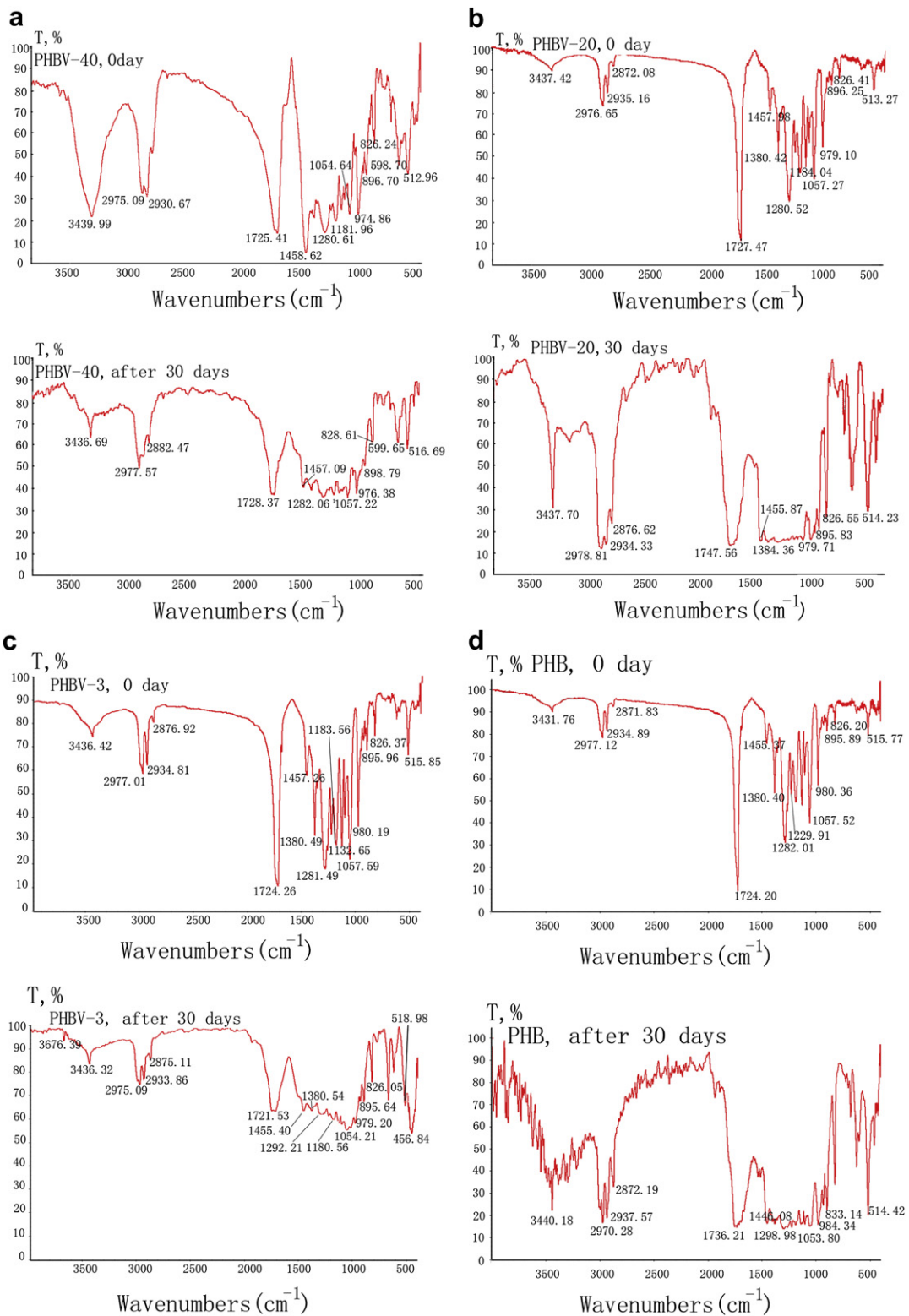


Fig. 5. The FT-IR spectra of different PHA samples before and after composting: a) PHBV-40; b) PHBV-20; c) PHBV-3; d) PHB; e) P(3HB,4HB).

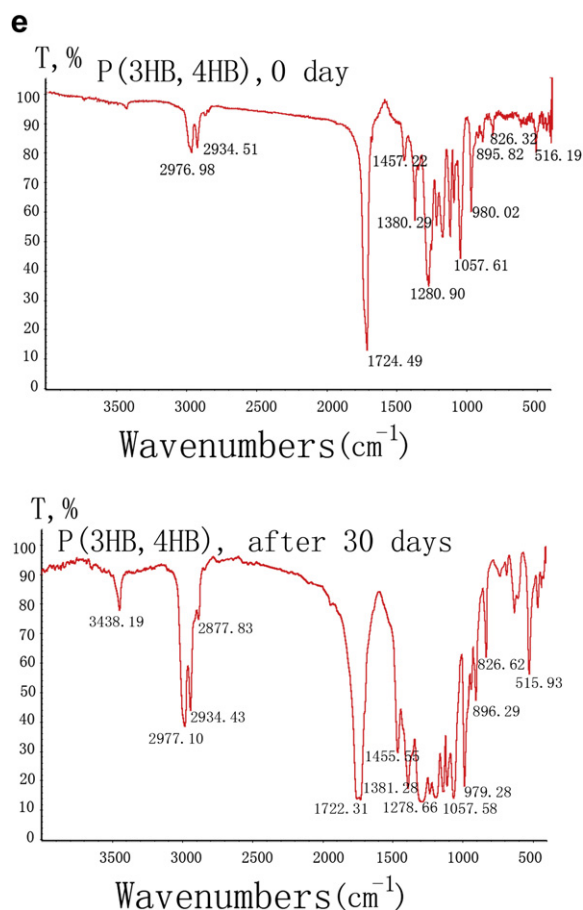


Fig. 5. Continued

(Waters 2414 Refractive Index Detector and Waters 1515 Isocratic HPLC Pump, USA).

3. Results and discussion

The appearance changes of PHA films during the composting were observed by digital camera and the photos are shown in Fig. 3. It can be seen that the size of all the PHA films with different chemical structures became smaller and smaller with the increase of composting time. After 10 days of composting, all the PHA films had lost their integrated appearance, with PHBV-40 and P(3HB,4HB) apparently degrading faster than the others. After 20 days, only 50% of the film could be found for all the samples. The degraded materials were either digested by the microorganisms or broken into such small fragments that no film residuals could be found in the compost. More material loss and a greater appearance change can be taken to reflect a faster degradation rate. Based on the appearance variations in Fig. 3, the order of biodegradation rate can be preliminarily determined as follows: PHBV-40 \approx P(3HB,4HB) > PHBV-20 > PHBV-3 > PHB.

The micro-morphology of PHA films with different chemical structure retrieved regularly from the composts was characterized by scanning electron microscope. The

results are shown in Fig. 4. It can be seen from the SEM images that the surfaces of PHA films were smooth before degradation. After 10 days of composting, the surfaces of all PHBV films were eroded and the surfaces became rough. The film surfaces of PHBV-40, PHBV-20, P(3HB,4HB) were seriously eroded and a network of cavities formed on the surface of PHBV-20. However, no cavities appeared on the surfaces of PHBV-3 and PHB. After 20 days of composting, many cavities were formed for all PHA samples. In fact, by that time all PHA films were almost disintegrated, as shown in Fig. 3. After 30-days it is difficult to see the cavities and there are only some PHA fragments. The surface morphology of PHB after 30 days of composting was very like that of PHBV-20 after 10 days of degradation. The order of biodegradation rate determined from the SEM photos was the same as that determined by the digital photos (Fig. 3).

From the above observations, both the apparent morphology and also the micro-morphology of PHA films illustrated that PHAs with different chemical structure had different biodegradation rates. Of all the PHAs, PHB had the most regular structure, which gives it the highest crystallinity [17]. As we know, the microbial degradation would occur initially in the amorphous region and, therefore, PHB had the lowest biodegradation rate. When HV structure was introduced into the PHB, the structure regularity was

damaged, resulting in a lower crystallinity of the PHBV. [18] More HV content would make PHBV have lower crystallinity [17]. Therefore, the biodegradation rate of PHBV-40 was fastest, while PHBV-3 was lower. The introduction of 4HB can also make the copolymer lower in crystallinity, [19] therefore, the biodegradation rate of P(3HB,4HB) was almost the same as PHBV-40.

The FT-IR spectra of PHAs with different structures before composting and after 30 days of degradation are shown in Fig. 5. It can be seen that PHA films before and after composting had similar absorption peaks in their FTIR spectra. In the 4000–3000 cm^{-1} region the bands ascribed to OH stretching appeared. After degradation, these bands (3385–3281 cm^{-1}) became obvious because more hydroxyl and carboxylic groups had been formed.

In the region of 3100–2800 cm^{-1} , bands at 2977–2975 cm^{-1} , 2935–2930 cm^{-1} , 2882–2871 cm^{-1} were attributed to CH_3 stretching vibration, CH_2 anti-symmetric vibration and CH_3 symmetric stretching vibration, respectively. The intensity and wave-number of these bands did not vary before and after degradation. The band at 1725–1724 cm^{-1} assigned to $\text{C}=\text{O}$ stretching vibration became wider after 30 days of biodegradation. The CH_3 deformation vibration, $\text{C}-\text{O}-\text{C}$ stretching vibration, and $\text{C}-\text{O}-\text{H}$ characteristic absorption were found at 1458–1455 cm^{-1} and 1384–1379 cm^{-1} , 1282–1280 cm^{-1} and 1184–1178 cm^{-1} . $\text{C}-\text{C}$ stretching vibrations were found in the range of 1000–800 cm^{-1} , which indicated the formation of the 21-helix

Table 3

The thermal decomposition temperatures of PHA before and after composting.

Samples	$T^a_{5\%}$ ($^{\circ}\text{C}$)	T^b_{max} ($^{\circ}\text{C}$)
PHBV-40	247	268
PHBV-20	234	264
PHBV-3	240	262
PHB	230	251
P(3HB,4HB)	268	287
Degraded PHBV-40	240	254
Degraded PHBV-20	182	247
Degraded PHBV-3	235	250
Degraded PHB	225	250
Degraded P(3HB,4HB)	238	256

^a 5% initial weight loss temperature ($T_{5\%}$).

^b maximum decomposition rate temperature (T_{max}).

structure of PHAs. [17] After degradation, the intensity of the above bands became weak.

The FTIR results also demonstrated that the biodegradation of PHA films was mainly caused by erosion by microorganisms and the erosion originated from the surface layer then spread gradually to the interior of the PHA films, while the chemical structure of residual PHA was not changed during the degradation process. A similar mechanism for composting degradation of PHBV was reported by Luo et al [20].

TGA is a commonly employed approach to evaluate the thermal stability and thermal decomposition behavior at various temperatures by measuring the onset degradation temperature ($T_{5\%}$) at which 5% mass loss occurs, the temperature for the maximum degradation rate (T_{max}) and char residue. The TG curves of PHAs with different chemical structures are shown in Fig. 6 and the relevant data are listed in Table 3.

From Fig. 6 and Table 3, it can be seen that PHBV-40 started to decompose at 247 $^{\circ}\text{C}$ at which it lost the end group from the main chain or ester change. After that, the weight loss increased quickly and the maximum degradation rate occurred at 268 $^{\circ}\text{C}$. The initial and the maximum decomposition temperatures for PHBV-20, PHBV-3, PHB, P(3HB,4HB) were 234 $^{\circ}\text{C}$, 240 $^{\circ}\text{C}$, 230 $^{\circ}\text{C}$, 268 $^{\circ}\text{C}$, and 264 $^{\circ}\text{C}$, 262 $^{\circ}\text{C}$, 251 $^{\circ}\text{C}$, 287 $^{\circ}\text{C}$, respectively, indicated that they had different thermal stability.

After 30 days of composting, the thermal decomposition temperatures for most PHA samples were shifted to lower temperatures. For example, the T_{max} was lowered to 254 $^{\circ}\text{C}$ for PHBV-40, 247 $^{\circ}\text{C}$ for PHBV-20, 250 $^{\circ}\text{C}$ for PHBV-3 and 256 $^{\circ}\text{C}$ for P(3HB,4HB), respectively. It can be concluded that, although the chemical structure of residual PHA was

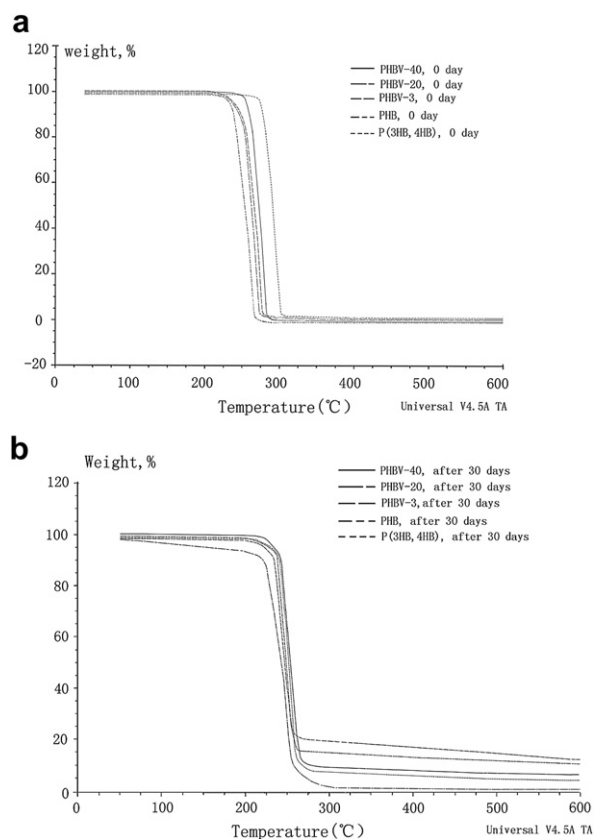


Fig. 6. TG curves of PHA films before (a) and after degradation (b).

Table 4

The number-average molecular weight of PHA before and after composting.

Sample	Mn before degradation $\times 10^4$ g/mol	Mn after degradation $\times 10^4$ g/mol	Decrease percentage %
PHBV-3	40.4	20.9	48.3
PHBV-20	32.4	15.7	51.5
PHBV-40	32.4	14.4	55.6
PHB	24.0	18.1	24.6
P(3HB, 4HB)	44.6	10.1	77.4

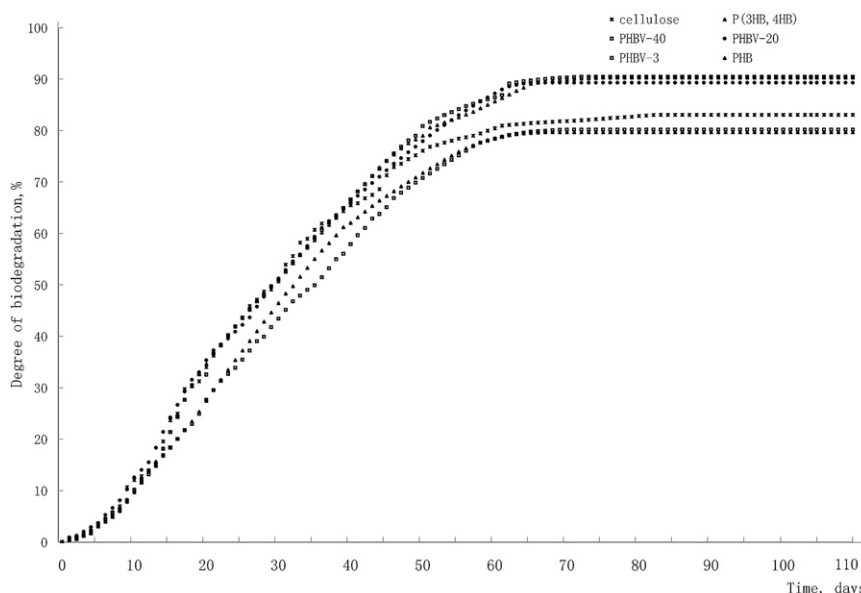


Fig. 7. The Biodegradation curves of PHAs.

not changed, the molecular weight of PHA had been greatly decreased, which made the biodegraded PHA have lower thermal stability than the untreated ones.

The number-average molecular weights (M_n) of PHA before and after composting are listed in Table 4. It can be seen that M_n of PHA decreased after degradation. The molecular-weight decrease percentages of PHBV-3, PHBV-20, PHBV-40, PHB and P(3HB,4HB) were 48.3%, 51.5%, 55.6%, 24.6%, 77.4% respectively.

Fig. 7 showed the biodegradation curves of PHBV-40, PHBV-20, PHBV-3, PHB, P(3HB,4HB) and cellulose in a controlled composting condition at 58 °C according to ISO 14855-1. It can be seen that PHBV-40, PHBV-20, PHBV-3, PHB, P(3HB,4HB) and cellulose started to degrade after 5 days of induction. PHBV-20 had the fastest speed of biodegradation in the first 15 days. The biodegradation curves of all the samples tended to level off after 70 days. The ultimate degrees of biodegradation of PHBV-40, PHBV-20, PHBV-3, P(3HB,4HB), PHB were 90.5%, 89.3%, 80.2%, 90.3% and 79.7%, respectively, after 110 days of biodegradation according to ISO 14855-1 using cellulose as the reference material whose degree of biodegradation was 83.1%. That is to say, the relative degrees of biodegradation of PHBV-40, PHBV-20, PHBV-3, P(3HB,4HB), PHB were 108.9%, 107.5%, 96.5%, 108.7% and 95.9%, respectively, relative to cellulose as a reference material.

According to ISO 17088:2008, the relative degree of biodegradation of a truly biodegradable material should not be less than 90% [21]. Therefore, based on the above results, PHBV-40, PHBV-20, PHBV-3, P(3HB,4HB) and PHB were all biodegradable materials. The order of biodegradation degree was PHBV-40 > P(3HB,4HB) > PHBV-20 > PHBV-3 > PHB. The reason for this difference in biodegradability of PHAs was that the crystallinity of the copolymer was decreased with the increase of HV and 4HB content. The amorphous part degrades faster than the crystalline part.

Similar results were reported by Chen et al. [22] and Wang et al. [23].

4. Conclusions

PHAs with different molecular structures, PHBV-40, PHBV-20, PHBV-3, PHB and P(3HB,4HB), were biodegradable under controlled composting conditions. The order of biodegradability was P(3HB,4HB) \approx PHBV-40 > PHBV-20 > PHBV-3 > PHB, which correlated well with the HV and 4HB content. The chemical structure of PHAs was not changed, but the thermal stability and the molecular weight were greatly decreased during the composting. The biodegradation of PHAs occurred by enzyme catalyzed erosion from the surface to the interior.

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