Biodegradation (2012) 23:127–132
DOI 10.1007/s10532-011-9492-y

ORIGINAL PAPER

# Study on the enzymatic degradation of PBS and its alcohol acid modified copolymer

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Received: 16 December 2010 / Accepted: 13 June 2011 / Published online: 6 July 2011


**Abstract** Enzymatic hydrolytic degradation of polybutylene succinate (PBS), poly(polybutylenesuccinate-co-1,4-cyclohexane dimethanol) (PBS/CHDM) and poly(polybutylene succinate-co-diglycolic acid) (PBS/DGA) in mixed solvent of tetrahydrofuran (THF) and toluene was examined. Lipase was used as catalyst to degrade polymers with molecular weight of more than 100,000, and the molecular weight of products ranged from hundreds to thousands. Thermal decomposition temperatures of all products were below 250°C. The degradation products of both PBS/CHDM and PBS/DGA showed two melting points at about 85 and 99°C. Mass spectrometry (MS) was employed to obtain the molecular weight of oligomers extracted from the products, which proved to be low-polyesters with the molecular weight of less 1,000. The butanediol (BDO) monomer was found in PBS/CHDM degradation product for the first time.

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**Keywords** Lipase · Degradation · PBS · Cyclic oligomer · Linear oligomer

**Abbreviations**

| | |
|---|---|
| PBS | Polybutylene succinate |
| PBS/CHDM | Poly(polybutylene succinate-co-1,4-cyclohexane dimethanol) |
| PBS/DGA | Poly(polybutylene succinate-co-diglycolic acid) |
| SA | Succinate |
| BDO | Butanediol |
| CHDM | 1,4-Cyclohexane dimethanol |
| DGA | Diglycolic acid |

## Introduction

The depolymerization of biodegradable polymers by enzymes has attracted researchers' great interest (Uyama and Kobayashi 2002; Himanshu Azim et al. 2006; Shuai et al. 1999; Rebeca Marcilla et al. 2006), a serial of biodegradable materials have developed as an approach to solve the increasing problems in plastics waste management. In recent years, aliphatic polyesters play the dominant role in biodegradable polymers and several research about the polyesters degradation by hydrolyses (lipases) have been reported (Soledad Marque's-Calvo et al. 2006; Vidal et al. 2007; Tokiwa and Calabia 2007;

Massardier-Nageotte et al. 2006; Abou-Zeid et al. 2004). Polymer with a supple and hydrophilic chains proved it has the biodegradability. During the polyesters catalyzed hydrolysis reaction lipase usually plays the main role (Suming Li et al. 2003; Kurokawa et al. 2008; Chen et al. 2004; Ronkvist et al. 2009; Feng He et al. 2005; He et al. 2003), and they were degraded to oligomers, which may be more suited for the repolymerization. This process can achieve to recycle carbon energy sources. Recently, the polymers were degraded by enzymes which be obtained a series of good results.

At present, there are several reports about poly ($\varepsilon$-caprolactone) and poly(lactic acid) (PCL/PLA) copolymers degradation by enzymes. With a long time, they can be degraded to oligomer in pH 7.6 phosphate buffer solution (Lenglet et al. 2009). Interestingly, the research about biodegradation of poly(butylene adipate) (PBA) seems to be especially active, which probably due to it's high degradation rate. Several studies concerning the degradation of PBA by enzymes or microorganisms have been confirmed (Okajima et al. 2003). The most common representative of this family of biopolymers, (PBS-co-PBA) can be degraded by different types of enzymes (Rizzarelli and Impallomeni 2004). In 100 days, immersing the films of polybutylene succinate-co-adipate (PBSA), PCL and PBS in lipase solutions at 37°C and pH 7.0, the lipase can succeed to enzymolysis the oligomers to monomers (Hoshino and Isono 2002).

However, the degradation of high molecular weight PBS and its copolymer by enzyme has not been reported. In this article, with simplified experimental device, we succeed to enzymolysis PBS, PBS/CHDM and PBS/DGA with high molecular weight to the yellow oligomers. This makes it hopeful to achieve the recycling of polyester raw material.

## Experiment

### Materials

Polybutylene succinate (PBS), binary copolymer of PBS and 1,4-cyclohexane dimethanol (PBS-co-CHDM), and binary copolymer of PBS and diglycolic acid (DGA) are synthesized in the laboratory (Min et al. 2008).

*Solvent* toluene, tetrahydrofuran (THF), chloroform ($CHCl_3$) are analytically pure, purchased from Xi'an Chemical Reagent Co. Ltd. Immobilized Candida lipase [CA: Novozym435] 10,000 plu/g, were purchased from Japan Vinorelbine Co. Ltd. all reagents were used directly without further purification.

### Equipments

Nicolet NEXUS-470 IR spectrometer (USA Nicolet Corporation) were used to determine the chemical structure of polymer before and after degradation; HT3-515GPC (U.S. Waters Corporation) was used to determine molecular weight of polymer before and after degradation; MALDI-TOF-MS (Micromass Company in British) was used to analyze the degradation products. The temperature of melting point ($T_m$) of the polymers and the degradation products of polymers were measured with a modulated DCS (DSC 2920, TA Instrument).Thermal degradation and the resulting weight loss of the samples were obtained by thermogravimetric analysis TGA (TG 209C, Germany NETZSCH Corporation).

### Preparation of polyester

By adopting SA, BDO, 1,4-CHDM, DGA as raw material, and Ti $(OiPr)_4$ as catalyst, PBS and copolymers with different ratio were obtained.

PBS were synthesized as following: SA: BDO (mol:mol = 1:1) were added into the three-necked flask with nitrogen flow, then Ti $(OiPr)_4$ was added, the reactant were heated to melt in the oil bath, 180°C and dehydrated for 1 h, until the volume of dehydration and the amount of the basic theory of dehydration were equal. Then vacuum degree was controlled below 6.5 Pa, continued to heat up to 230°C and reacted for about 1 h, the expect product were got. Products were purified with $CHCl_3$, vacuum drying for 24 h. Modified copolymers were synthesized in the same way.

PBS/CHDM [SA:BDO:CHDM (mol:mol:mol = 10:10:1)] and PBS/DGA [SA:BDO:DGA (mol:mol:mol = 10:10:1)] were prepared by the same method as mentioned above.

### Polymers degradation conditions

THF (20 ml), distilled water, CA enzyme (0.08 g), PBS (0.5 g) were added into a 100 ml three-necked

flask and stirred at 60°C for 12 h under atmospheric pressure, then toluene were added, volume ratio (v/v) of THF and toluene were 2:1. After continuously reacting under the same conditions for 12 h, CA enzymes were filtered and recycled, solvent was recycled by evaporation, and degradation products were obtained finally and were purified further by THF, purified products were analysis by MS.

PBS/CHDM and PBS/DGA were degraded by the same method as mentioned above.

## Discussion

### GPC analysis

Table 1 show that after the process of are catalyzed and hydrolyzed by enzymes, the polyesters has been degraded to a series of low-polyesters, and the molecular weight of them were declined from more than 100,000 to tens of thousands. Products with molecular weight of 40,000 could be found in PBS degradation products, but the highest molecular weight of the degradation products of PBS/CHDM and PBS/DGA was only about 10,000, so PBS/CHDM and PBS/DGA is better than PBS in degradation.

In contrast, PBS/CHDM and PBS/DGA degradation products with low molecular weight were more than the PBS degradation products, degradation products of PBS/CHDM had the minimum number average molecular weight of 380, and more obviously, oligomers with many kinds of molecular weights appeared in the degradation products.

**Table 1** GPC spectra of polyesters and products of polyesters

| Molecular weight of polymers Mn × $10^4$ | | | Molecular weight of product Mn | | |
|---|---|---|---|---|---|
| PBS | PBS/CHDM | PBS/DGA | PBS | PBS/CHDM | PBS/DGA |
| | | | 4,7488 | 14,728 | 10,431 |
| | | | 2,441 | 1,329 | 1,124 |
| 10.00 | 10.46 | 11.6 | 1,400 | 878 | 600 |
| | | | 8,68 | 527 | |
| | | | | 380 | |

### Thermal properties of the polymers and degradation products

Table 2 and Fig. 1 show that although the melting point (Tm) of degradation products declined, it could maintain 111°C, this was because the PBS degradation products contain a polyester whose molecular weight is more than 40,000. However $T_m$ of PBS/CHDM and PBS/DGA degradation products were below 100°C. Most notably they both had two endothermic peaks which were $T_m$ peak of the oligomers, the peak at 85°C referred to the oligomers with molecular weight ranging from hundreds to thousands while the peak at 99°C referred to the oligomers with the molecular weight of 10,000.

Changes of the melting point could prove that degradation of modified PBS was better than PBS. This was due to that PBS/CHDM contained six-membered ring which resulted it more space between molecular chains, thus exposure of ester bond, which was beneficial for enzymes attacking; Ether bond ($–CH_2–O–CH_2–$) in DGA of the PBS/DGA was hydrophilic, thus prompting the hydrolysis reaction.

**Table 2** Temperature of melting for polymers

| | PBS | PBS/CHDM | PBS/DGA |
|---|---|---|---|
| Tm (°C) | 115.5 | 107.5 | 106.1 |

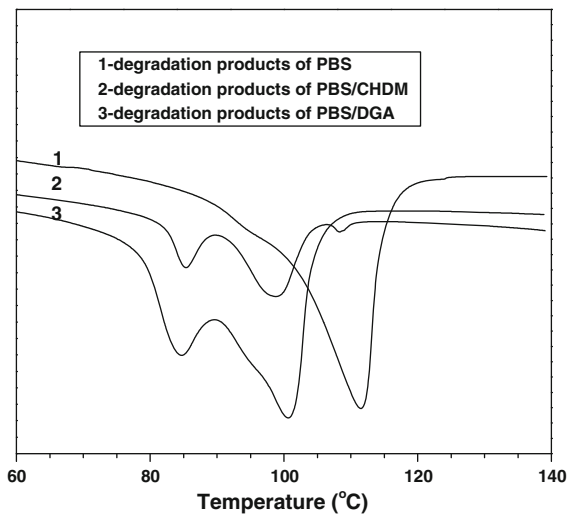



**Fig. 1** The temperature of melting point ($T_m$) of the degradation products of polymers were measured with a modulated DSC

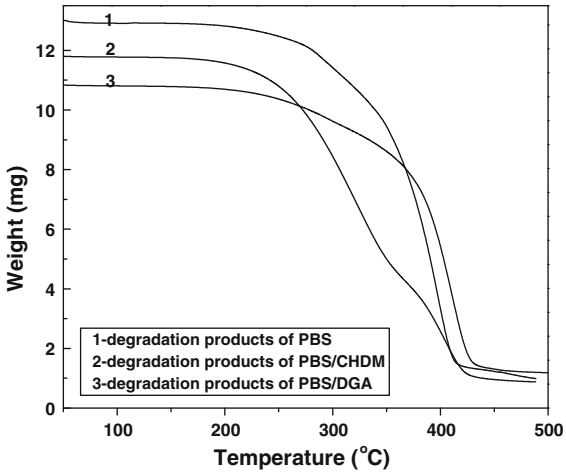



**Fig. 2** Thermal degradation and the resulting weight loss of the products were obtained by thermogravimetric analysis TGA

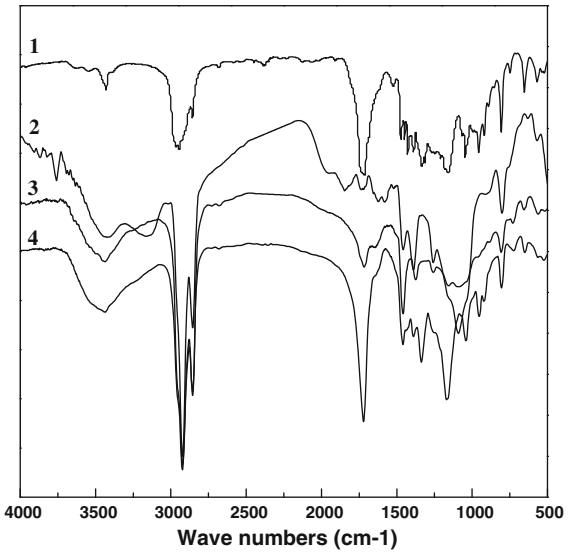



**Fig. 3** FT-IR spectra of *1* PBS, *2* degradation product of PBS, *3* degradation product of P(BS-co-CHDM), *4* degradation product of P(BS-co-DGA)

Figure 2 shows the weight of the degradation products of PBS and the copolymer began to loss below 250°C and the total weight was lost at around 500°C, which proved that the thermal decomposition temperatures ($T_{ds}$) were low, which indicated that the molecular weight of degradation products was not very large. Weight of degradation products of PBS and PBS/DGA began to loss at around 215°C correspondingly the decomposition of oligomers, while the temperature of degradation products of PBS/CHDM was 200°C, since degradation products of PBS/CHDM contented more oligomers, resulting in better degradation than PBS and PBS/DGA.

## IR spectra of PBS and degradation products of PBS and PBS copolymer

The degradation products of PBS and PBS copolymer were analyzed by FT-IR. Fig. 3 shows the ester bond of PBS had an absorption peak in the 1850–1650 $cm^{-1}$, but absorption peaks of PBS and PBS/CHDM degradation products were weak, indicating that their carbonyl content decreased and a large number of ester bonds of PBS and PBS/CHDM were hydrolyzed and destroyed.

But in the PBS/DGA degradation products the sharp carbonyl peak appeared, which was due to more acid content of PBA/DGA and the degradation products were mostly terminated by carboxyl. The peak at 2,925 $cm^{-1}$ referred to that of C–H in the methyl and methylene, the peak of C–H of degradation products was sharper than that of PBS, this was because the PBS molecular chain could form intermolecular hydrogen bonds, but the intermolecular hydrogen bonds of degradation products with low molecular weight were very weak, so the C–H peak was very sharp.

An absorption peak in the 3400–3200 $cm^{-1}$ could be observed, because the content of low polyesters in the degradation products increased, thus increasing the concentration of hydroxyl, arousing the association of carbonyl compounds, also the stretching vibration absorption peak of O–H shifted to lower wave numbers, all these leaded to a wide and strong absorption peak.

## MS spectra of purified products of PBS and PBS copolymer

Purified products of polyester were analyzed by MS, Fig. 4 gave the information of oligomers structures and the chemical structure was shown in Table 3, indicating that linear oligomers of PBS degradation products were all dimers, trimers and tetramers. BDO and succinic anhydride monomers were found in the PBS/CHDM degradation products. There were also a small amount of methanol succinate cyclohexanedicarboxylic esters and cyclic trimers. Degradation

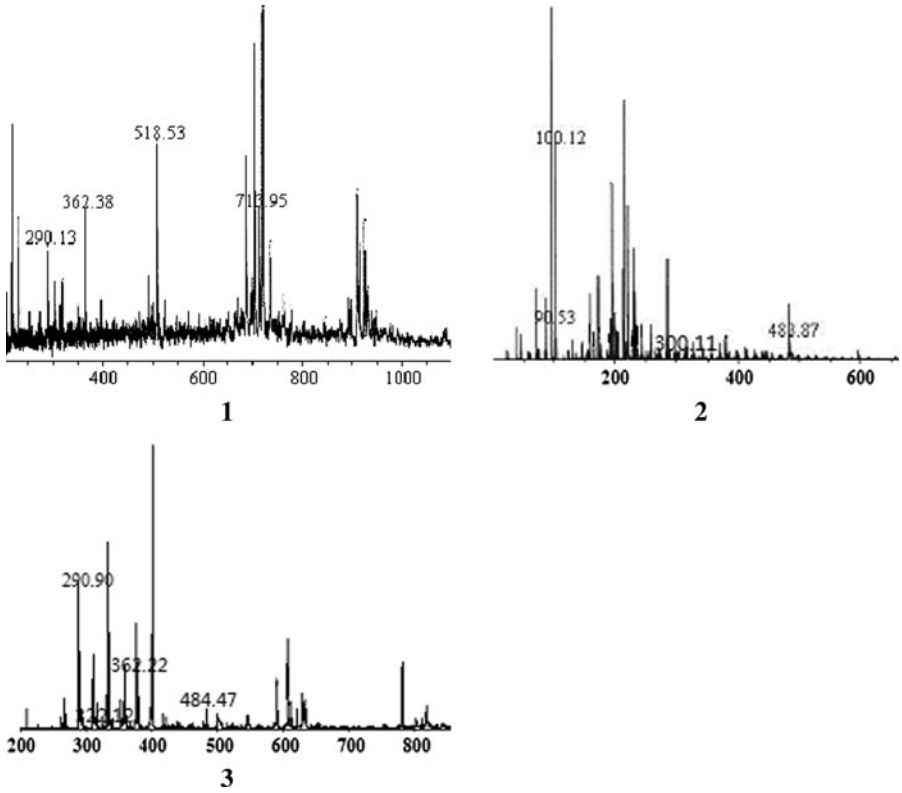
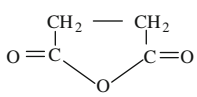
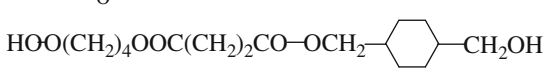



**Fig. 4** MALDI-TOF MS spectrum of the degradation products of (1-PBS, 2-PBS/CHDM, 3-PBS/DGA)

**Table 3** Chemical structure of the degradation products was analyzed by MALDI-TOF MS

| 1 | m/z | Structure | Symbol |
|---|---|---|---|
| | 290 | HOOC $CH_2$ $CH_2COO(CH_2)_4OOCCH_2CH_2COOH$ | $BS_1{\cdot}SA_1{}^+$ |
| | 362 | $HO[OC\ CH_2\ CH_2COO(CH_2)_4]\ _2OH$ | $BS_2{}^+$ |
| | 518 | $HO[OC\ CH_2\ CH_2COO(CH_2)_4]\ _3OH$ | $BS_3{}^+$ |
| | 714 | $HO[OC\ CH_2\ CH_2COO(CH_2)_4]\ _4OH$ | $BS_4{}^+$ |
| 2 | 90 | $OHCH_2(CH_2)_2CH_2OH$ | $BD^+$ |
| | 100 |  | Succinic anhydride |
| | 300 |  | $BS_1CHDM_1{}^+$ |
| | 484 |  | Cyclic $BS_3{}^+$ |
| 3 | 291 | $HOOC\ CH_2\ CH_2COO(CH_2)_4OOCCH_2CH_2COOH{\cdot}H$ | $BS{\cdot}SA{\cdot}H^+$ |
| | 322 | $HO(CH_2)_4OOCCH_2OCH_2COO(CH_2)_4OH$ | $BDO{\cdot}DGA{\cdot}BDO^+$ |
| | 362 | $HO[OC\ CH_2\ CH_2COO(CH_2)_4]\ _2OH$ | $BS_2{}^+$ |
| | 484 |  | Cyclic $BS_3{}^+$ |

The number of PBS degradation products is 1

The number of PBS/CHDM degradation products is 2

The number of PBS/DGA degradation products is 3

products of PBS/DGA included linear dimers, cyclic trimers and a little of BDO·DGA·BDO.

In a word, PBS/CHDM could obtain the optimized degradation among the three types of polyesters, oligomers of PBS/CHDM contained a few monomers, because PBS/CHDM contained six-membered ring, double helix chains of PBS were damaged, resulting in more space between molecular chains, more extent of chains stretching and ester bond exposure which was beneficial for enzymes attacking.

## Conclusion

The polymers were degraded by enzymes, a series of low-polyesters were obtained, and the molecular weight of products mainly ranged from hundreds to thousands. The degradation products of both PBS/CHDM and PBS/DGA showed two Tm were located at 85 and 99°C, PBS/CHDM had the best degradation in the three types of polyester, oligomers of PBS/CHDM contained a little of monomers.

**Acknowledgments** The author thanks National Natural Science Foundation of China and Zhejiang Wenzhou Research Institute of Light Industry for their financial support. This project was supported by the Graduate Innovation Fund of Shaanxi University of Science and Technology and Key Laboratory of Auxiliary Light Chemical Industry in Shaanxi Province.

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